Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop:
November 2-4, 1987,
Toronto, Ontario

Compte rendu des communications do quatorième atelier annuel sur la toxicitè aquatique:
du 2-4 novembre, 1987,
Toronto, Ontario

Editors/Éditeurs

A.J. Niimi and K.R. Solomon

May 1988

Mai 1988

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1607

Rapport technique canadien des sciences halieutiques et aquatiques n° 1607

Available from Department of Fisheries and Oceans
Bayfield Institute (GLLFAS)
Burlington, Ontario L7R 4A6

S'adresser au ministerè des Pêches et Océans
Institut Bayfield (LGLPSA)
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Minister of Supply and Services Canada 1988

Cat. No. Fs 97-6 1607E
ISSN 0706-6457

Correct citation for this publication:

Niimi, A.J. and K.R. Solomon (eds) 1988. Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop: November 2-4, 1987, Toronto, Ontario. Can. Tech. Rep. Fish. Aquat. Sci. No. 1607.

Ministère des Approvisionnements et Services Canada 1988

No. de cat. Fs 97-6 1607E
ISSN 0706-6457

On devra référer comme suit à cette publication:

Niimi, A.J. et K.R. Solomon (eds) 1988. Compte rendu des communications du quatorzième atelier annuel sur la toxicite aquatique: du 2 au 4 novembre 1987, Toronto, Ontario. Rapp. tech. can. sci. halieut. aquat. no. 1607.

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ACKNOWLEDGEMENTS

The organizing committee thanks the following and acknowledges the sponsors for their financial and/or other support.

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Many persons worked to make the workshop a success. We extend our thanks to all of them. We also thank the authors, session chairpersons and the participants for their contributions and to the projectionists. Madge Barclay gave freely of her time at the registration desk and we owe her special thanks. We also extend our gratitude to the staff of the Delta Chelsea Hotel, Toronto, for their co-operation and efforts for the workshop.

Special thanks are due to Mr. Charles Caccia for his thought-provoking and informative presentation at the banquet.

The proceedings were completed with the support of the Department of Fisheries and Oceans, Ottawa.

PREFACE

This report is the Proceedings of the Fourteenth Annual Aquatic Toxicity Workshop, held in Toronto, Ontario from November 2-4, 1987.

The Fourteenth Annual Aquatic Toxicity Workshop was one of a continuing series of annual Workshops in Canada on aquatic and environmental toxicology, covering topics from basic aquatic toxicology to applications in environmental monitoring, setting of regulations and guidelines, and the development of water quality criteria. These Workshops emphasize an informal exchange of ideas and knowledge on the topics among interested persons from industry, governments and universities. They provide an annual focus on the principles, current problems and approaches in aquatic toxicology. These Workshops are run by an incorporated National Steering Committee, and the proceedings are published with the support of the Department of Fisheries and Oceans.

The theme of the 1987 Workshop was "Laboratory-to-field Extrapolation Technology". Papers and posters were solicited on topics relating to research in aquatic toxicology. Seventy-three papers (51 oral and 21 poster) were presented and the topics covered a number of important areas: Toxicology of organic substances, effects of acid precipitation, toxicity of metals in fish, aquatic ecotoxicology, water quality and MISA, techniques in aquatic toxicity testing, biochemical indicators of toxicity and chemical contaminants and fish tumors.

EDITORS COMMENTS

This volume contains papers, abstracts or extended abstracts of all presentations at the Workshop. An author index and a list of participants are also included. The papers and abstracts were reviewed by steering committee members but were not subjected to external review. Comments on any aspects of individual contributions should be directed to the authors. The proceedings of the fish tumor symposium are being edited by C.D. Metcalfe and it is planned that they will be published as a refereed symposium proceedings by the American Fisheries Society.

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COST OF INACTION AND THE ENVIRONMENT

The Honorable Charles Caccia, House of Commons, Parliament Buildings, Ottawa, Ontario.

Today in Canada the politics of the environment, compared to ten years ago, offers a picture of bustling activity: media coverage is intensive, the public is keen, politicians feel the pressure, bureaucracies are asked to deliver. At the root of all this is a public increasingly preoccupied not only about today but also about tomorrow. On no other policy sector do politicians refer so persistently and frequently to 'present and future generations', as they do when taking about the environment. Therefore industry can expect increased and persistent public pressure on national governments and U.N. agencies to clean-up existing operations, to manage waste intelligently, to aim at zero discharge, to ensure sustainable development.

How humanity can sustain itself and continue to develop was the theme studied, and reported on in April this year, by the World Commission on Environment and Development. The path of sustainable development, as outlined in 'our common future', is a possible way of ensuring economic development without environmental degradation.

In order to make policy decisions that will send us down the path of sustainable development, our policy-makers require information that, at present, either does not exist or is, at best, sketchy. The cost of inaction is central to this theme. The cost of action to protect the environment is readily available to policy makers and advisors with cost-benefit analyses which favour the short term. For example when an acid rain control program for the United States is proposed, industry quickly produces the costs, to themselves and the consumer of their product, of implementing such controls.

The cost of inaction continues to be an elusive quantity; addressed by relatively few. It is imperative that means be found to determine the growing environmental debt and the cost of not acting to prevent further abuses.

In testimony to the Special Committee on Acid Rain, Thomas Crocker, an economist at the University of Wyoming, concluded that: "assuming that the impact of acid deposition is to reduce forest productivity by 5 percent per annum, then the annual losses to commercial timber in Canada would be $197 million in 1981 dollars. To this annual loss of $1.29 billion (in 1981 dollars) should be added for disruption to recreation and wildlife habitat values. These estimates which are very crude should be treated as 'ball-park' estimates that may be useful in the rank-ordering of receptor catagories."

How do we improve our estimates of the long-term economic, social and environmental cost of not doing anything, the cost of inaction?

We have created an astonishing array of pollutants, natural and man-made, in copious quantities. In some cases evidence of the damage is visual; dead fish, dying maples trees or smog, in others it is not; contaminated groundwater, reproductive failure or genetic alteration. Defending cause-effect relationships between specific pollutants and ecological damage is, as we all know, very difficult.

How rapidly is the damage occurring? The rate at which man is altering the natural environment is a key variable in determining the economic and societal costs of not cleaning up the source of pollution doing the damage, and the time frame in which action must take place.

How much then is the cost of inaction? Literally, the multi-billion dollar question. Up to this point the data required for the determination of the cost of inaction have been in fields of biological sciences; forestry, fisheries, medicine, botany. The crux of the matter is translating the biological or ecological losses into a dollar figure. It is this transfer from science to economics that has traditionally short-changed the environment. All to often, particularly in cost-benefit analyses, many ecological values are rated as 'intangibles' because the traditional, discount-fixated economist cannot derive a unit cost for wilderness, aesthetics, non-commercial wildlife, clean air, etc. There is change gradually emerging, there is a faint beginning in the transfer of thought from science to economy, the development of an environmental economics.

To scientists, like yourselves, I put the following proposition: is it time to launch a dialogue between yourselves, the data collectors, and economists, the data manipulators, so as to generate the ammunition for politicians, the data users. The challenge is to develop a methodology of translating scientific findings into cost of inaction data. This does not mean economics will become mandatory for all biologists! But it could mean changing the manner scientists collect and transmit data to the non-scientific community and in ensuring they are understood. It is time to broaden the audience that receives and understands scientific findings.

To tell Cabinet one-third of our forests are suffering from pollution stress is not enough to stimulate political will because biology cannot overcome the economic arguments in favour of the lesser cost of inaction. Being able to inform Cabinet that pollution removes hundreds of millions of dollars per year from the economy would catch attention and generate support. But we are still far away from the day when the cost of inaction, placed in the hands of decision-makers will give them a powerful tool with which to put the cost of clean-up, or of prevention, into proper perspective. We have a lot of homework to do.

I invite you comments and seek your help.

CURRENT SWEDISH RESEARCH ON ORGANOHALINES IN KRAFT MILL WASTES

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ABSTRACT

The biological effects of effluents from pulp industries, especially from bleaching processes on aquatic ecosystems were studied in a joint biological/ chemical project. A receiving body of water for pulp bleach plant effluents at the Gulf of Bothnia was chosen for the three year study. Near the effluent outlet the fish biomass was low, and the species composition of the fish community had changed. Perch (*Perca fluviatilis* L.) exhibited reduced reproduction and disturbed physiology up to 10 km from the outlet. The effluent also affected the diversity, biomass and distribution of invertebrates and plants. To further study the effects, fish and benthic organisms were exposed in the laboratory to sediment from the polluted area and to various mixtures of bleach plant effluents. The level of extractable organic chlorine (EOCl) of the perch decreased along a gradient from the effluent outlet towards the open sea. In sediment outside the Swedish coast, the level of EOCl and the occurrence of compounds related to bleach mill effluents indicated distribution of such substances over large areas.

In recent years Swedish forest industry has tested several techniques that reduce the discharge of chlorinated material. As a response to new environmental requirements, this work is still in progress and new measures have been taken. Oxygen bleaching, lower chlorine charge alone or in combination with chlorine dioxide addition and careful process optimization are useful means to meet the new requirements. To reduce the discharges further, new process technology has to be introduced, e.g. the Prenox process, membrane filtration of effluents and partial or complete recycle of bleach effluents.

RÉSUMÉ

Les effets biologiques d'effluents de l'industrie des pâtes et papier, notamment des processus de blanchiment, sur les écosystèmes aquatiques ont été étudiés dans le cadre d'un programme mixte de recherche biologique et chimique. Un plan d'eau récepteur d'effluents d'une usine de blanchiment de pâtes et papier, dans le golfe de la Bothnie, a été choisi pour cette étude de trois ans. À la sortie de l'effluent, la biomasse des poissons était à un has niveau et la composition spécifique était modifiée. Jusqu'à 10 km en aval de la sortie des effluents, la perche (*Perca fluviatilis* L.) se reproduisait moins bien et avait des troubles physiologiques. Les effluents nuisaient également à la diversité, à la biomasse et à la distribution des invertébrés et des plantes. Afin de parfaire l'examen des effets, des poissons et certains organismes benthiques ont été exposés en laboratoire à des sédiments provenant du secteur pollué ainsi qu'à différents mélanges d'effluents de l'usine de blanchiment. La concentration en composés organochlorés extractibles chez la perche diminuait selon un gradient à partir de la source de l'effluent jusqu'à la haute mer. Dans les sédiments au large des côtes de la Suède, la concentration en composés organochlorés et la fréquence d'observation de composés liés aux effluents de l'usine de blanchiment ont permis de se faire une idée de la distribution de ces substances dans une grande région.

Au cours des dernières années, l'industrie forestière de la Suède a testé différentes techniques visant à réduire le rejet de composés chlorés. Afin de répondre à de nouvelles exigences écologiques, l'industrie poursuit ses travaux et de nouvelles measures ont été prises. Le blanchiment à l'oxygène, l'abaissement de la charge en chlore uniquement ou en combinaison avec l'addition d'hypochlorite ainsi qu'une optimisation soignée du processus constituent des moyens utiles pour parvenir à se conformer aux nouvelles exigences. Pour réduire davantage les effluents, il faut introduire une nouvelle technologie, p. ex., le processus Prenox, la filtration sur membrane des effluents et la recirculation partielle ou totale fes effluents de blanchiment.

THE EFFECTS OF OIL SANDS WASTEWATER ON FISH: A TAINTING STUDY

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ABSTRACT

Oil sands wastewaters taken from two different storage areas of the Syncrude Canada Operations in Fort McMurray, were used to determine their potential to taint fish. Hatchery rainbow trout were exposed to various concentrations of catchment basin water and tailings pond water for 60 to 72 h at the Fort McMurray oil sands plant. Fish were then removed from the treatments, prepared (head and fins removed, and eviscerated), vacuum packed in air-tight plastic bags and shipped to Diversified Research Laboratories Limited in Toronto for sensory evaluation. Significant perceivable differences were recorded for fish exposed to the higher concentrations of the two different wastewaters. Another series of experiments were designed and executed to determine rate of uptake while exposed to high concentrations of oil sands wastewaters and rate of depuration after fish were tainted by the wastewater from the oil sands plant. Results obtained from other exposures are being analyzed presently.

Tissue and bile were removed from rainbow trout exposed to the various treatments during the study. Initial analyses have revealed conjugated alkylated phenols in the bile. Further analyses are being carried out presently in order to determine other chemicals that might be responsible for tainting of rainbow trout by oil sands wastewater.

RÉSUMÉ

Le potentiel d'eaux usées pour gâter le poisson a été mesuré. Les eaux usées provenaient de l'exploitation du sable bitumineux de la Syncrude Canada à Fort McMurray et provenaient de deux aires différentes de stockage. Des truites arc-en-ciel d'élevage ont été exposées à différentes concentrations d'eau d'un bassin re retenue d'un bassin de décantation pendant 60 à 72 h, à l'usine de sable bitumineux de Fort McMurray. Les poissons étaient ensuite soustraits au traitement, conditionnés (tête

et nageoires enlevées, éviscération), emballés sous vide dans des sacs de plastique étanches et expédiés à *Diversified Research Laboratories Limited* à Toronto pour l'évaluation sensorielle. D'importantes différences ont été notées chez les poissons exposés aux concentrations les plus fortes des deux différentes sortes d'eaux usées. Une autre série d'expériences a été faite pour déterminer le taux d'absorption des poissons exposés aux concentrations les plus fortes d'eaux usées ainsi que le taux de dépuration du poisson gâté par les eaux usées. Les résultats obtenus lors d'autres expositions sont en cours d'analyse.

Des tissus et de la bile de truites arc-en-ciel exposées aux différents traitements ont été prélevés en cours d'étude. Les analyses préliminaires ont indiqué la présence d'alkylphénols conjugués dans la bile. D'autres analyses sont en cours et devraient permettre de déterminer d'autres composés chimiques susceptibles d'avarier la truite arc-en-ciel.

ECOTOXICOLOGY HAZARD ASSESSMENT: OPTIMIZATION OF THE BIOLOGICAL TESTING

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ABSTRACT

In an hazard assessment perspective, it is possible to improve the ecotoxicological interpretation generated from standard biological testing. It is generally accepted that information like effluent persistence, assimilation capacity of the receiving water or tolerance of the biological system, are necessary to complement the toxicity data. These aspects are presently neglected. Our aim is to implement a scheme of biological screening tests coupled to modification screening procedures in order to assess a more representative evaluation of the effects of an effluent. These procedures were initiated with a pulp and paper effluent where, despite the primary toxicity data, modifications of the effects at the effluent level (degradation, fractionation), receiving water level (dilution water) and biological level (recovery) were studied. Our results tend to demonstrate that this type of information is more useful for a management purpose: it could become a powerful screening and hazard assessment approach capable of predicting ecotoxicological effect of waste water discharge in lotic or lentic aquatic ecosystem.

RÉSUMÉ

Dans une perspective d'évaluation des risques, il est possible d'améliorer l'interprétation écotoxicologique des tests biologiques standard. Il est généralement admis que les renseignements comme la persistance des effluents, la capacité d'assimilation des plans d'eau récepteurs ou la tolérance des systèmes biologiques constituent des compléments nécessaires aux données de toxicité. À l'heure actuelle, ce sont des aspects négligés. Notre objectif est de mettre en place une batterie de tests de dépistage biologique couplés à des méthodes de dépistage par modification des effets

afin d'obtenir une image plus juste des effets d'un effluent. On a commencé à tester ces méthodes avec un effluent d'usine de pâtes et papier utilisé pour l'examen des effets au niveau de l'effluent (décomposition, fractionnement), à celui du plan d'eau récepteur (eau de dilution) et au niveau biologique (récupération) indépendamment des données primaires de toxicité. Nos résultats tendent à montrer que ce type de renseignements est plus utile que les autres dans une perspective de gestion: cette méthode pourrait devenir un puissant instrument de dépistage et d'évaluation des risques qui nous permettrait de prévoir l'effet écotoxicologique du rejet d'eaux usées dans un écosystème aquatique lotique ou lentique.

EFFECTS OF GREAT LAKES GENERATING STATION THERMAL DISCHARGE ON DEVELOPMENT AND SURVIVAL OF ROUND WHITEFISH EGGS - A COMBINED LABORATORY AND FIELD STUDY

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ABSTRACT

Thermal requirements of developing round whitefish (*Prosopium cyclindraceum*) eggs were determined in a laboratory study to assess effects from thermal discharges. Eggs were subjected to 16 combinations of constant and fluctuating temperatures (1.7 to 10°C) for the entire incubation period (December-April). Response surface models were used to relate baseline temperature and temperature change to egg survival or development times. Over the 1984-85 winter, a test of these models was conducted in Lake Ontario near Pickering Nuclear Generating Station. Field incubators were placed (5-7 m depth) at 6 locations from the Rouge River (3 km west of Pickering) to Thickson Point (12 km east of Pickering). Fertilized eggs were placed in the incubators in December and samples recovered by divers at five intervals from January to April. From thermograph daily mean temperatures, the laboratory models predicted the developmental stage and survival expected at each site on collection days. Correspondence between observed and predicted development was especially good in the later stages. Predicted dates for median hatch averaged 2.3 d later than observed (total range ± 10 d). Predicted survivals were higher than observed at all sites. Predictions agreed with observed results in that survival progressively decreased over the 4 sites from Thickson Point to the Pickering 5 m location. A substantial portion of the mortality at the Pickering 5 m site resulted from a 3°C temperature elevation. High mortality associated with the western sites (Pickering 7 m, Rouge River) was not the result of temperature and may reflect higher sediment loadings.

RÉSUMÉ

Les exigences sur le plan thermique d'oeufs de ménomini rond (*Prosopium cyclindraceum*) ont été déterminées en laboratoire afin d'évaluer les effets du rejet d'eau chaude. Les oeufs ont été soumis à 16 combinaisons de température constante et variable (1.7 à 10°C) pendant toute la durée d'incubation (décembre-avril). Des modèles ont été utilisés pour établir un rapport entre la température nominale ainsi que les variations de température et la durée de survie ou de développement des oeufs. Au cours de l'hiver 1984-1985, ces modèles ont été testés dans le lac Ontario près de la centrale nucléaire de Pickering. Des incubateurs de terrain ont été disposés (5-7 m de profondeur) à 6 endroits entre la rivière Rouge (3 km à l'oeust de Pickering) et Thickson Point (12 km à l'est de Pickering). Les oeufs fécondés ont été placés dans les incubateurs en décembre et des échantillons ont été prélevés par des plongeurs à 5 intervalles différants de janvier à avril. Á partir des températures moyennes quotidiennes données par les thermogrammes, les modèles de laboratoire ont donné des prévisions de l'étape de développement et de la survie à chaque station les jours de prélèvement. La correspondance entre les observations et les prévisions du développement a été remarquable avec les dernières étapes de développement. Les dates médianes prévues d'éclosion dépassaient en moyenne les dates d'éclosion observées d'environ 2.3 j (plage totale ± 10 d). Les taux prévus étaient supérieurs à ceux observés à toutes les stations. Les prévisions concordaient avec les observations en ce sens que la survie diminuait progressivement de l'une à l'autre des 4 stations comprises entre Thickson Point et la station de Pickering à 5 m. Une importante partie de la mortalité à la station 5 m de Pickering tenait à un réchauffement de l'eau de 3°C. La forte mortalité observée dans les stations de l'ouest (Pickering 7 m, Rivière Rouge) n'était pas causée par la température de l'eau, mais pouvait résulter de charges sédimentaires supérieures.

KINETICS OF THE DESPORPTION OF MERCURY FROM SELECTED FRESHWATER LAKE SEDIMENTS AS INFLUENCED BY CITRATE

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ABSTRACT

The formation of mercury-organic complexes can take place in Hg contaminated aquatic environments. Since the majority of the Hg in aquatic ecosystems is sorbed by sediments and the concentration of organic acid is relatively high in the sediment-water interface, the interaction between the sediment-bound mercury and organic acids may increase the dispersion rate of Hg from the sediment to the water phase by forming mercury-organic complexes. The objective of this experiment was to study the kinetics of the Hg desorption from selected freshwater sediments as influenced by citrate and pH. The citrate solutions were adjusted to pH 7.0 with 0.1 M NaOH for the kinetic study and were not adjusted for the study of the combined effect of pH and varying citric acid concentrations. The results show that Hg desorption, in the citrate solution at pH 7, obeyed the multiple first-order kinetics, a fast desorption of Hg from the sediments in the first hour followed by a much slower desorption. The amount of the Hg desorbed from the sediments increased with the increasing citrate concentration from 0 to 0.01 *M* and varied with the nature of the sediments. The increase of the Hg desorbed became more pronounced when the concentration of citrate was 10^{-3} *M* or higher. The desorption of Hg was negligible when the pH of the citric acid solutions was not preadjusted regardless of the citric acid concentrations studied. This is attributable to the coagulation of the sediments particles at low pHs. Therefore, the influence of citrate on the desorption of Hg from the sediments was highly pH dependent.

RÉSUMÉ

Des complexes de composés organiques et de mercure peuvent apparaître dans les milieux aquatiques contaminés par le Hg. Comme la majeure partie du Hg des systèmes aquatiques est sorbée par les sédiments et que la concentration en acides organiques est assez élevée à l'interface eau-sédiments, les réactions entre le mercure captif des sédiments et les acides organiques peuvent accroître le taux de dispersion du Hg dans la phase aqueuse par formation de complexes de composés organiques avec le mercure contenu dans les sédiments. L'objectif de cette expérience était l'etude de la cynétique de la désorption du Hg contenu dans certains sédiments d'eau douce, à un certain pH et dans un milieu modifié par l'citrate. Les solutions d'citrate ont été ajustées au pH 7.0 avec du NaOH 0.1 M pour l'étude de la cynétique; elles ne l'ont pas été pour l'étude de l'effet du pH à plusieurs concentrations d'acide citrique. Les résultats montrent que la désorptionn du Hg en solution d'citrate à pH 7 se faisa conformément aux règles de la cynétique multiple de premier ordre, soit une désorption rapide du Hg contenu dans les sédiments qui est suivie par une désorption considérablement ralentie. La quantité de Hg désorbé augmentait avec l'accroissement de la concentration en citrate (de 0 à 0.01M) et différait selon la nature des sédiments. L'augmentation de la quantité de Hg déso:bé devenait très

prononcée quand la concentration en citrate atteignait 10^3 M et plus. La désorption du Hg était négligeable quand le pH des solutions d'acide citrique n'était pas d'abord ajusté, peu importe les concentrations d'acide citrique essayées. On attribue ce fait à la coagulation des particules sédimentées à faible pH. Ainsi, l'effet de l'citrate sur la désorption du Hg contenu dans des sédiments dépend beaucoup du pH.

INTRODUCTION

Organic materials can interact with inorganic Hg to form complexes in aquatic environments (Schindler et al. 1972; Lindberg and Harriss 1974; Miller 1975; Thanabalsingam and Pickering 1985a). The reactions are likely to occur in the interstitial water where the levels of inorganic Hg and organic materials are relatively high compared with the bulk water. The formation of the Hg-organic complexes can transfer the sediment-bound Hg to more soluble forms (Cline et al. 1973; Miller et al. 1975).

It has been shown that humic and fulvic acids can strongly bind the inorganic Hg through various functional groups such as carboxyl, hydroxyl and carbonyl groups (Strohal and Huljev 1970; Schnitzer 1971; Miller 1975; Kerndoff and Schnitzer 1980; Thanabalasingam and Pickering 1985a). Ramamoorthy and Kushner (1975) believed that the binding is a result of complexation between the organics and Hg rather than of physical adsorption on surface of the organics. By using radiotracer to study the interaction between Hg (II) and humic acid, Strohal and Huljev (1970) found that Hg (II) was very strongly bound to humic acid but the process was reversible. However, the absorbed Hg cannot be displaced by a wide variety of cations. Reimers and Krenkel (1974) found that carboxyl, amino and hydrosulfyl functional groups of the long alkanes can strongly bind the inorganic Hg.

Some of the low-molecular-weight organics are relatively abundant in freshwater ecosystems and have specific functional groups. The influence of these low-molecular-weight organics on the desorption of the sediment-bound Hg under varying pH conditions still remain obscure. Therefore, the objective of this study was to investigate the kinetics of the desorption of Hg from selected sediments as influenced by citrate at varying pHs.

MATERIALS AND METHODS

Freshwater sediment samples were obtained from the Buffalo Pound, Katepwa and Pasqua Lakes in the Qu'Appelle River basin in Saskatchewan, Canada. The sediments were sampled using an Ekman dredge and stored in a cold room at 4±0.5°C. The pH and Eh of the sediments were measured, respectively, by glass/calomel and platinum-/Ag/AgCl combination electrodes in a 1-g sediment/50-mL distilled water suspension at 25°C.

Each wet sediment sample (8 g of oven dried weight basis) was suspended in 120 mL deionized distilled water. One hundred and sixty mg of Hg, as $Hg(NO_3)_2.H_2O$, was dissolved in 80 mL distilled water and the Hg solution was then added to the sediment suspension and equilibrated for 24 h at 25°C in a constant temperature shaker. Each sediment suspension at the end of the reaction was divided into 8 parts and centrifuged for 20 min at 1600*g*. The total Hg remaining in the supernatant was determined to calculate the amount of Hg adsorbed by the sediments.

The sediment after centrifugation was dispersed in a series of 50 mL citrate solutions (pH=7.0), which contained citrate concentrations ranging from 0 to 10^{-2} *M* and were adjusted to pH 7.0 with 0.01 *M* NaOH, in a 125-mL Erlenmeyer flask. The sediment suspensions in the flasks at the end of each reaction period was flushed to a Hg° trap (Lodenius et al. 1983) with N_2 gas. The amounts of Hg found in the Hg° traps were in the nanogram range, which accounts for <0.01% of the Hg released to the solution phase, indicating that the Hg volatilized from the system was negligible. The sediment suspensions were then filtered through as 0.45 μm membrane filter at the end of each desorption period. Aliquots of the filtrates were digested and analyzed for total Hg by the cold vapor technique at a wavelength of 253.7 nm (U.S. E.P.A. 1974) using a uv mercury analyzer. The amounts of Hg in the interstitial water of the sediment samples, which is less than 0.3 μg Hg per g sediment, before dispersion in the citrate solutions were calculated and subtracted from the final results. The pH and Eh of the sediments were measured by Dissolved Oxygen Meter (Extech, Boston, Mass.)

Selected properties of the lake sediments studied are presented in Table 1. The hydrous oxides in the sediment samples were determined by extraction with acidified hydroxylamine hydrochloride (Chao and Zhou 1983). The organic carbon contents in the sediment samples were determined by the dry combustion method (Tiessen et al. 1981).

RESULTS AND DISCUSSION

Fig. 1 shows the rate curves for Hg desorption from the lake sediments in 0, 10^{-4}, 10^{-3}, and 10^{-2} *M* citrate at 4 and 25°C. The results indicated that the Hg desorption from all three lake sediments obeyed the multiple first order kinetics. A fast desorption process occurred in the first hour, followed by a much slower desorption process. The rate constants of each desorption process (Table 2) show that the rate constants of the fast desorption increased with increasing the concentration of citrate. When the citrate concentration was increased from 0 to 10^{-2} *M*, the Hg desorption rate increased by 3.6, 7.6, and 8.9 times for Katepwa, Buffalo Pound and Pasqua Lake sediments, respectively. Buffalo Pound Lake sediment had the slowest rate constant and Katepwa Lake sediment had the highest among the three sediments studied (Table 2). The Hg desorption rate constants (Table 2) appeared to be related to the contents of organic carbon and hydrous oxides of Al, Fe, Mn and Si of the sediments and the mean depth of the lake (Table 1).

Table 1. Selected properties of Buffalo Pound, Katepwa, and Pasqua Lake sediments.

	Buffalo Pound	Katepwa	Pasqua
pH (±0.1)	7.9	7.6	7.6
Eh (mv, ±20)	480	425	450
Organic carbon (g/kg)	16.8±1.4	46.6±1.2	46.8±1.7
Extractable hydrous oxides (g/kg)			
Al	0.70±0.01	1.24±0.02	1.24±0.02
Fe	4.58±0.01	7.31±0.11	7.25±0.12
Mn	0.27±0.01	0.85±0.01	0.82±0.01
Si	1.32±0.02	2.13±0.08	2.10±0.08
Mean lake depth (m)	3.0	14.4	5.9

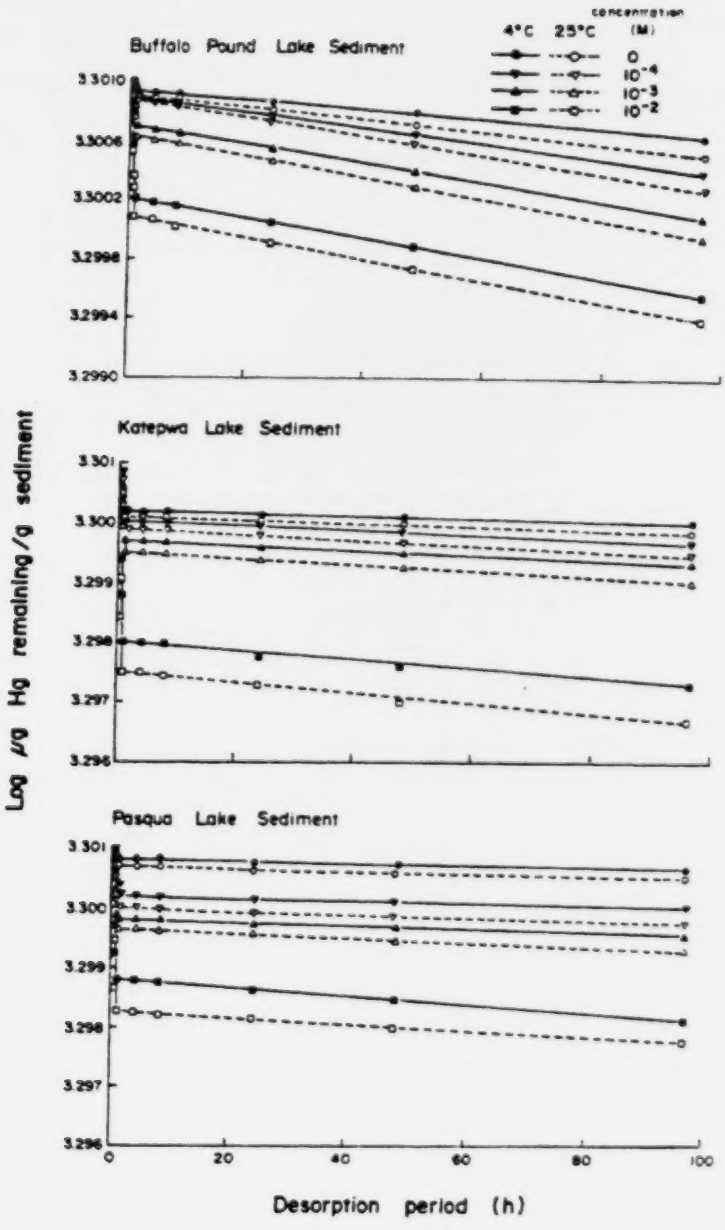

Fig. 1. The rate curves of the Hg desorption from the lake sediments in 0, 10^{-4}, 10^{-3}, and 10^{-2} M citrate solutions at 4 and 25°C. The pH of the citrate solution was adjusted to 7.0 with 0.01 M NaOH.

After the initial fast desorption process was completed in the first hour, the rates of the subsequent Hg desorption reactions decreased by two to three orders of magnitude in the reaction period of 2-96 h. The rate constants of the slow desorption process, however, still increased with increasing citrate concentration (Table 2).

Table 2. The rate constants of the fast and slow desorption processes of Hg from the lake sediments as influenced by citrate.

Citrate (M)	Reaction[1] steps	Rate constants $x10^5$ (per h)	
		4°C	25°C
	Buffalo Pound Lake sediment		
0	I	11±1	13±1
	II	0.30±0.03	0.39±0.03
10^{-4}	I	15±1	16±2
	II	0.50±0.04	0.62±0.05
10^{-3}	I	34±4	41±4
	II	0.62±0.05	0.67±0.07
10^{-2}	I	84±7	94±8
	II	0.65±0.07	0.72±0.07
	Katepwa Lake sediment		
0	I	84±9	92±8
	II	0.15±0.03	0.24±0.04
10^{-4}	I	98±12	113±12
	II	0.37±0.04	0.44±0.04
10^{-3}	I	133±18	152±18
	II	0.35±0.04	0.48±0.06
10^{-2}	I	303±24	353±26
	II	0.74±0.08	0.84±0.09
	Pasqua Lake sediment		
0	I	25±5	34±6
	II	0.16±0.01	0.22±0.02
10^{-4}	I	86±7	103±9
	II	0.20±0.02	0.30±0.04
10^{-3}	I	126±12	139±15
	II	0.26±0.03	0.38±0.05
10^{-2}	I	222±19	276±21
	II	0.73±0.08	0.54±0.05

[1] I = The 0-1 h reaction period.
II = The 1-96 h reaction period.

Although the inorganic Hg can be strongly bound by sediment components (Reimers and Krenkel 1974; Wang et al. 1985a, b), some of the sediment-bound Hg could still be released upon changes of limnological conditions, such as pH, Eh, dissolved oxygen content, ligand concentration and Hg concentration in the solution-sediment system.

Table 3. The pH, Eh, and dissolved oxygen of the sediment suspensions and the release of Hg and the dissolution of hydrous oxides from the sediments after 24 h desorption period at 25°C.

Citrate (M)[1]	Susp. pH	Eh (mv)	Dissol. O_2 µg/g water	Hg rel. µg/g sediment	Al	Dissolved oxide Fe	Mn	Si
						(µg/g sediment)		
				Buffalo Pound Lake sediment				
0	7.9	425	4.8	1.3±0.1	nd[3]	nd	nd	nd
10^{-4}	8.0	450	4.8	1.4±0.1	nd	0.1±0.0	0.1±0.0	nd
10^{-3}	7.6	460	3.0	2.6±0.2	nd	0.5±0.1	0.3±0.0	nd
10^{-2}	7.4	471	1.3	5.2±0.4	nd	8.3±1.0	2.1±0.2	nd
				Katepwa Lake sediment				
0	7.9	450	3.6	4.5±0.4	nd	nd	nd	nd
10^{-4}	7.7	447	3.5	5.8±0.6	nd	0.1±0.0	0.1±0.0	nd
10^{-3}	8.1	434	3.0	7.7±0.4	0.4±0.1	0.5±0.1	0.3±0.1	nd
10^{-2}	8.2	396	1.7	17.1±1.2	1.6±0.1	16.0±0.2	5.4±0.1	nd
				Pasqua Lake sediment				
0	7.8	470	3.5	3.1±0.1	nd	nd	nd	nd
10^{-4}	7.9	450	3.3	4.7±0.1	nd	0.1±0.0	0.3±0.0	nd
10^{-3}	8.0	410	3.0	6.9±0.2	0.1±0.0	0.2±0.0	0.4±0.0	nd
10^{-2}	8.1	400	1.8	13.4±0.4	0.8±0.1	10.0±0.5	4.1±0.3	nd

1 The initial pH of the citric acid solutions was adjusted to 7.0 with 0.01 M NaOH.

2 The precision of dissolved O_2 measurements was ±0.1 µg/g water.

3 Not detectable.

The fast desorption process reflected the redistribution of the Hg in the adsorbed and soluble form immediately after the citrate solution was introduced to the sediment.

The data in Table 3 show that as the citrate concentration was increased from 0 to 10^{-2} M, the pHs of the suspensions generally increased slightly. Except for the Eh of the Buffalo Pound Lake sediment, the Eh and dissolved oxygen content, on the other hand, decreased as the citrate concentration was increased (Table 3). A substantial increase in Hg release was observed in all three sediments when initial pH of the citrate solution was 7.0 and its concentration was increased from 0 to 10^{-2} M. The stability constant of Hg (II)-citrate complex is $10^{-10.9}$ (Sillen and Martell 1971). The enhancement of Hg released from the sediments is partly attributed to the formation of Hg(II)-citrate complexes. The amounts of soluble Al, Fe, and Mn oxides were also increased with the increasing citrate ligand concentration (Table 3), indicating that some of the poorly crystalline Al, Fe and Mn oxides were dissolved through the formation of metal-citrate complexes. Since the poorly crystalline oxides can strongly adsorb inorganic Hg (Rogers et al. 1984; Thanabalsingam and Pickering

1985b; Wang et al. 1985b), part of the Hg released from the sediment may be attributed to the dissolution of the poorly crystalline oxides. In addition, the data on pH, Eh, and dissolved oxygen (Table 3) indicate that some other chemical and physical changes such as redox processes in the sediment systems could also have occurred. The Hg released from the sediments may be the combined effects of all the chemical and physical changes in the system. The desorption of Hg from the sediments appeared to be slightly faster at 25°C than at 4°C (Table 2). However, the effect of temperature on the Hg desorption rate was not substantial, if the experimental errors were taken into account.

The data in Table 4 show that the Hg released from the sediments decreased drastically when the pH of citric acid solution was not adjusted drastically before addition to the sediments regardless of the citric acid concentrations studies. The decrease of Hg from sediments with the decreasing pH was also observed by Jackson et al. (1980) and Zvonzrev and Zyrin (1983). Jackson et al. (1980) attributed the decrease in Hg released from the sediments at lower pH to the exceptionally strong covalent bonding between the Hg and humic matter, whereas Anderson (1967) indicated that the decrease in Hg release was due to the decreasing solubility of the humate-Hg complex at lower pH. The data obtained in the present study indicate that H^+ may interfere with the Hg release by retarding the complex formation between the sediment-bound Hg and citrate ligands and/or by coagulating the sediment suspensions which can significantly reduce the surface area and the amount of adsorbed Hg exposed to the citrate ligand. The coagulation of the sediment suspensions was visually observed immediately after the addition of citrate solutions with pH <4.1.

CONCLUSIONS

The Hg desorption from the lake sediments as influenced by citrate ligands obeyed the multiple first order kinetics. A fast desorption process occurred in the first hour, followed by a much slower desorption process. The rate of the Hg release from the sediments increased with increasing citrate ligand concentration especially at 10^{-3} M or higher. The increase in the rate and amount of Hg released from the sediments is attributed to the combined effects of complexation by Hg by citrate ligand, decreasing suspension Eh and dissolved oxygen, and increasing dissolution of the poorly crystalline oxides of Al, Fe, and Mn. The Hg desorption was negligible when the pH of the citric acid solutions were not preadjusted to 7.0 regardless of the citric acid concentrations studied. Temperature had little effect on the Hg released from the sediments by citrate ligand in the temperature range of 4 to 25°C.

ACKNOWLEDGEMENTS

This study was supported by the Natural Sciences and Engineering Research Council of Canada - Strategic Grant 32629 - Huang.

Table 4. Mercury released from the lake sediments as influenced by citric acid solutions at varying pHs in a 24 h reaction period at 25°C.

Citric acid conc. (M)	pH	Suspension[1] pH	Hg released (µg/g sediment)
		Buffalo Pound Lake sediment	
0	7.0	7.9	1.3±0.1
		5.8[2]	7.91.1±0.1
	2.0	6.2	0.2±0.0
10^{-4}	7.0	8.0	1.4±0.1
	4.1[3]	7.9	0.6±0.1
10^{-3}	7.0	7.6	2.6±0.2
	3.0[3]	7.1	0.3±0.1
10^{-2}	7.0	7.4	5.2±0.4
	2.7[3]	4.3	0.2±0.0
		Katepwa Lake sediment	
0	7.0	7.6	4.5±0.6
	5.8[2]	7.6	4.5±0.4
	2.0	6.6	0.3±0.1
10^{-4}	7.0	7.7	5.8±0.6
	4.1[3]	7.5	1.6±0.1
10^{-3}	7.0	8.1	7.7±0.4
	3.0[3]	7.1	0.6±0.1
10^{-2}	7.0	8.2	17.1±1.2
	2.7[3]	6.4	0.3±0.1
		Pasqua Lake sediment	
0	7.0	7.8	3.1±0.6
	5.8[2]	7.6	3.0±0.4
	2.0	6.8	0.2±0.1
10^{-4}	7.0	7.9	4.7±0.6
	4.1[3]	7.6	1.1±0.1
10^{-3}	7.0	8.0	6.9±0.4
	3.0[3]	7.1	0.4±0.1
10^{-2}	7.0	8.1	13.4±1.2
	2.7[3]	6.7	0.2±0.1

1 The precision of suspension pH measurements was ±0.1.
2 The pH of the deionized distilled water before adjustment.
3 The pH of the citric acid solutions at varying concentrations before adjustment.

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A SURVEILLANCE PROGRAM TO ASSESS THE HEALTH OF AQUATIC ECOSYSTEMS EXPOSED TO CONTAMINANTS

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ABSTRACT

An assessment framework has been developed for the evaluation of the effects of aquatic contaminants on ecosystem health. The framework draws heavily on concepts from fisheries stock assessment and classifies the response of fish populations in terms of population characteristics such as growth, reproduction and larval performance. The framework does not require an extended data set over a period of years and has been shown to be sensitive during field validation. Several general response patterns have been identified for preliminary diagnosis of ecosystem health. The framework has been applied successfully in a three year combined field and laboratory assessment of a chain of lakes exposed to mixed metal mining waste. General limitations and applicability to other field studies shall be discussed.

RÉSUMÉ

Un plan a été conçu poue l'évaluation des effets de contaminants aqautiques sur l'equilibre des écosystèmes. Le plan emprunte à des notions d'évaluation des stocks pour la pêche et permet de classer la réaction de populations de poissons en termes de caractéristiques démographiques telles la croissance, la reproduction et la performance des larves. Le plan ne nécessite pas la constitution d'une importante base de données sur plusieurs années et il a été montré sur le terrain qu'il est sensible. Plusieurs types généraux de réactions ont été trouvés qui peuvent servir à un diagnostic provisoire de l'équilibre d'un écosystème. Le plan a été appliqué avec succès à une évaluation de trois ans, en laboratorie et sur le terrain, de lacs en chapelet exposés à des résidus mixtes d'extraction de minerai métallifère. Il sera question des grandes limites et de l'applicabilité du plan à d'autres études sur le terrain.

EXTENDED ABSTRACT

There is a need to develop inexpensive methods for evaluating contaminant impacts on aquatic ecosystems. This paper describes the rationale for sentinel selection and choice of surveillance level, as well as a simple, rapid, cost-effective mechanism for *in situ* assessment of toxicant impact on feral fish populations. The frame work is entitled Population Indicators of Sublethal Contaminants Effects on Suckers (PISCES), and assumes that changes in birth or death rates, or alterations in availability of food or habitat resources are associated with characteristic responses of sucker populations. The framework draws heavily on concepts from fisheries stock assessment and classifies the response of fish populations in terms of population characteristics such as growth, reproduction and larval performance. The framework does not require an extended data set over a period of years and has been shown to be sensitive to field validation. The responses have been grouped into five main response patterns based on population characteristics, including age distribution, growth, mean age, age to maturity, fecundity, egg size, catch per unit effort and condition factors. A major advantage of examining a wide range of population characteristics is that fluctuations which are not consistent with the overall general

response pattern can suggest areas for intensive, follow-up study. All patterns are distinguishable by examination of the mean age, fecundity and condition factors of populations, relative to those in a comparable, control population. Populations which are growing, reproducing and surviving at rates indistinguishable from the control population are considered to be free from adverse chemical effects.

The framework was tested and developed over the past three years while examining the effects of mixed metal mining water on growth, reproduction and survival of white suckers in northern Ontario. Feral suckers were collected from lakes with elevated levels of both copper (13-15 μg/L) and zinc (209-253 μg/L). Age of maturity of these suckers was between 4 and 6, and until 6 y of age there was no difference in length or weight of fish collected from control and contaminated lakes. After this age, contaminated fish were significantly smaller and shorter, and female suckers failed to exhibit significant increases in either length or weight after the age of maturity. Fish from contaminated lakes also exhibited decrease in egg size and fecundity, failed to show significant increases in fecundity with age, and exhibited an increased incidence of spawning failure. Suckers from the contaminated sites failed to show differences in mean age or condition factor.

Changes in suckers from contaminated sites corresponded well with a response pattern associated with a change in feeding habits. Failure of female fish to grow significantly after maturity and decreased energetic commitment to reproduction also suggested that the food base of the lakes was limiting the performance of female suckers. Additional work showed several major food groups were missing from the sediments of contaminated sites, and previous work suggested that sediments under water deeper than 5 m may be incapable of supporting macroinvertebrate fauna.

PISCES has several limitations, and is dependent upon selection and appropriate sampling of a comparable reference population. However, analysis of several studies showed that in each case, the conclusion offered by PISCES was identical to that of the study group after investment of intensive research efforts. The PISCES system has the potential to act as a tool for preliminary diagnosis of ecosystem impact and to suggest areas for intensive follow-up study. The system requires evaluation in areas with different stressors and contaminant combinations to fully evaluate its potential.

EFFECTS OF TWO PH CONDITIONS ON VITELLOGENESIS IN RAINBOW TROUT

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ABSTRACT

The acidification of lakes and rivers has been associated with the decline of fish populations. Laboratory experiments suggest that adult fish can survive at pH conditions encountered in the wild, yet field data show that acid stressed populations

suffer recruitment failure. We investigated the effect of pH exposure on production of the egg yolk precursor, vitellogenin (Vg), in rainbow trout. Vg, a glycolipophosphoprotein associated with calcium, may be indirectly measured by serum total phosphoprotein phosphorous (TPP) and serum calcium (SCa). Recently a homologous radioimmunoassay has been developed which offers greater precision and sensitivity. Mature rainbow trout were exposed to 3 pH conditions (4.5, 5.5 and control) for a period of 20 d during the months of September- October and November-December, 1986. Blood was sampled from individually labelled fish on days 0, 10 and 20 and analyzed for SCa, TPP and Vg.

Neither direct (Vg) or indirect indicators (SCa and TPP) show a significant effect at either acid condition after 20 d. However, the levels of these parameters were lower in pH 4.5 exposed fish and could indicate a lag in reproductive development at this low pH. The implications of this lag will be discussed, especially with reference to soft water (low Ca^{+2}) and acid conditions.

RÉSUMÉ

On a associé l'acidification des lacs et des rivières à la diminution du nombre de poissons. Des expériences en laboratoire semblent indiquer que des poissons adultes peuvent survivre aux pH trouvés dans la nature, et pourtant les données sur le terrain montrent un recrutement imparfait chez les populations stressées par des conditions acides. Nous avons étudié l'effet de l'exposition de poissons à différents pH sur la production du précurseur du vitellus, la vitellogénine (Vg), chez la truite arc-en-ciel. La Vg, une glycolipophosphoprotéine associée au calcium, peut être mesurée indirectement par le phosphore total des phosphoprotéines (PTP) et le calcium sérique (CaS). Il y a peu de temps, un dosage radioimmunologique très sensible et de très grande précision a été mis au point. Des truites arc-en-ciel à maturité ont été exposées à trois pH (4.5, 5.5 et pH témoin) pendant 20 d durant les mois de septembre et octobre ainsi que novembre et décembre 1986. Des échantillons de sang ont été pris chez des sujets marqués aux jours 0, 10 et 20 et ils ont été dosés pour la teneur en CaS, PTO et Vg.

Ni les indicateurs directs (Vg) ni les indicateurs indirects (CaS et PTP) ont indiqué d'effet important chez les sujets exposés au deux milieux acides pendant 20 d. Cependant les concentrations observées chez le sujets exposés au pH 4.5 étaient inférieures et pouvaient indiquer un retard dans la maturation sexuelle à ce pH. Les conséquences de ce retard seront analysées, notamment au regard des conditions en eau douce (peu de Ca^{+2}) et en condition acide.

STUDIES ON INTERACTIONS BETWEEN COMPONENTS OF ELECTROPLATING WASTES

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ABSTRACT

Interactions between metals representative of electroplating wastes (Cu, Cd, Zn, hexavalent Cr and trivalent Cr) were analyzed with two bioassays: the Microtox test and the protozoan test (*Colpidium campylum*). Significant differences between the sensitivity of the two organisms to toxicants were found. Dual and triple interactions between metals are observed and differences exist between the bacterium and the protozoa. So the estimation of the ecotoxicological risk of electroplating wastes cannot be based either on the chemical analysis only or on the results of only one bioassay.

RÉSUMÉ

Les interactions entre des métaux représentatifs des effluents de traitements de surface (Cu, Cd, Zn, Cr hexavalent et Cr trivalent) ont été étudiées a l'aide de deux tests biologiques: le test Microtox et un test protozoaire (*Colpidium campylum*). Des différentes significatives de sensibilité aux métaux ont été observées entre les deux organismes. Des interactions doubles et triples ont été mises en évidence et elles sont différentes selon le test biologique utillsé. Dans ces conditions, le risque écotoxicologique lié aux effluents de traitement de surface ne peut être estimé correctement sur la base d'une simple analyhse chimique ou sur le résultat d'un seul essai biologique.

INTRODUCTION

Industrial wastes have a very complex composition, and it is very difficult to estimate the toxicity of such waters on the basis of chemical analysis. Very often, interactions occur between the components and the resulting toxicity does not reflect at all the toxicity of each of the products which have been identified in the waste.

Electroplating wastes are a good model for the approach of interactions because highly toxic metals are present in the liquor (Cu, Cd, Ni, Zn and traces of hexavalent Cr); some other metals are less toxic or have not been studied yet (trivalent Cr). The nature of interactions between these metals have been well studied because very few bioassays are available for such extensive experiments.

Antagonism between Zn and Cd were found for many organisms: a ciliate protozoan (Dive et al. 1983), cultured algae and phytoplankton (Gingrich et al. 1984; Braek et al. 1980), crustacea (Thorp and Lake 1974; Negilski et al. 1981; Attar and Maly 1982), fishes (Eisler and Gardner 1973), and mammals (Nordberg et al. 1979; Prosi 1983). Some authors have found additive effects with *Photobacterium phosphoreum* (Dive et al. 1983) or synergistic effects with *Hormidum rivulare* (Say and Witton 1977). During their work with shrimp, Thorp and Lake (1974) had seen an antagonism of the two metals for low concentrations and an additivity for high concentrations.

Interaction found between Zn and Cu are hardly always a synergy (Sprague and Ramsay 1965; Eaton 1973; Sterrit and Lester 1980; Malins and Collier 1981; Wallace 1982; Lutton et al. 1984; Reinstein et al. 1984), and rarely an antagonism (Braek et al. 1980). Between Cu and Cd, synergistic effects were observed by Sprague and Ramsay (1965), Baldry (1977), and an antagonism by Bartlett et al. (1974). Stebbing and Santiago-Fandino (1983) found with *Campanularia flexuosa* (Hydrozoa) different interactions in term of the concentrations tested and the duration of experiments. Interactions of Cr with other metal are not well known. Baldy (1977) observed on the bacteria *Klebsiella aerogenes* an additivity of the effects of chromate and Cu and an antagonism between chromate and Cd. A synergy between Ni and Cu was found by Babich and Stotzky (1983) with Gram-negative bacteria and actinomycetes.

During the present work, we have studied the interactions between a few of the toxic metals present in electroplating wastes using two bioassays: the Microtox test (procaryotic organism) and the ciliate protozoan *Colpdium campylum* (eucaryotic organism). The significance of these interactions in the toxicity of the wastes must be studied to decide if the chemical analysis of effluents is sufficient to have a correct estimation of the ecotoxicological risk.

MATERIALS AND METHODS

Metals

The metals examined were Cu^{++}, Cd^{++}, $Cr207^{--}$, Zn^{++} and Cr^{+++}. Analytical salts (Merck) or atomic absorption spectroscopy standards (Baker) were used to prepare the standard solutions.

Bioassays

The Microtox test is based on the measurement of the decreased bioluminescence of the marine bacteria *Photobacterium phosphoreum* when toxic substances are added to the bacterial medium. Bioassays are carried out in a Microtox toxicity analyzer system (Beckman), at 20°C, after 30 min, as described in the Beckman Interim Manual (1982). Ten µL of bacterial suspension (1 mL of cold distilled water per flask of lyophilized bacteria) are diluted with 90 µL of 2% NaCl solution. After an initial measurement of light output (Io), 900 µL of test solution previously adjusted to 2% NaCl are added. The residual luminescence is measured again after 10 and 30 min. A control is run simultaneously to evaluate the physiological variation of luminescence expressed by the blank ratio BR where:

$$BR = \frac{It}{Io} \text{ for the control medium.}$$

Toxicity in the toxic medium is expressed by the ratio β of residual luminescence to initial luminescence, corrected for BR:

$$\beta = \frac{Io}{It \cdot BR}$$

Each test was run in triplicate using different bacterial reagents.

The ciliate bioassay using *C. campylum* was performed according to a previous work (Dive et al. 1982). The number of generations measured after 24 h cultivation was used as the biological response. Cultures were reared in a mineral medium ($CaCl_2.2H_2O$, 107 mg; NaCl, 14.5 mg; $NaNO_3$, 4.5 mg; $MgSO_4.7H_2O$, 75.7 mg; Na_2SO_4, 39.5 mg; $NaHCO_3$, 135 mg; H_2O s.a.f 1 L; pH 8.15). Lyophilized bacteria (*Escherichia coli* ATCC 11303, Sigma) were used as nutrients (Dive 1982; Vasseur et al. 1986).

Bioassays were carried out in polystyrene screw capped flasks (30 ml) at 28°C in the dark under the following conditions (Vasseur et al. 1986):

Dilution of the toxic substance(s) in culture medium . . .	4 mL
Suspension of *E. coli* (2.5 mg/mL) in culture medium. . .	0.2 mL
48 h culture of *C. campylum* s.a.f.	.2500 cells
Medium s.a.f..	5 mL

After 24 h incubation, the flasks were fixed with 1 mL of 5% glutaraldehyde and enumerated using a Coulter Counter (200 μm aperture). Results are expressed as the number of generations (NG) obtained during 24 h incubation (No = initial cell concentration = 500 mL; N = final cell concentration) by the relationship:

$$NG = \log (2) (N/No)$$

Experiments

Complete factorial experiments were conducted to examine the interactions. Interpretation of results was based on multiple regression analysis and graphic representation of the response surface as described by Bois et al. (1986) and Dive et al. (1986). An interaction is obvious if the combined effect of two substances is different from the sum of their individual effects when tested alone. Two substances are non-interactive if the combined effect if the sum of the individual effects.

The graphic representation is different for protozoan and bacterial tests. For bacterial assays, the toxicity values (-log β), plotted on the vertical axis, are higher when toxicity is increased (Fig. 1a). For the protozoan test, where the criterion of number of generations plotted on the vertical axis, the values are lower when toxicity is increased. Plotting of the toxic concentrations, on the horizontal axis, were also reversed to make it easier to read the results.

RESULTS

Acute toxicity of single metals.

Table 1 shows the CI50 values for Cd, Cu, Zn, and K bichromate on the two organisms. The results show similar range of CI50 for Cu and hexavalent Cr. The bacterium was more sensitive than the ciliate to Zn and less sensitive to Cd.

Effects of combined metals on *P. phosphoreum*.

Cd-Zn mixtures: At low dose (up to 0.375 mg/L), Zn increased the toxicity of Cd slightly (up to 2.4 mg/L), as shown in Fig. 1a. Only additive effects were observed for high concentrations of Zn (Fig. 1b).

Cd-hexavalent Cr mixtures: A synergistic effect occurred only at high concentration (30 mg/L) of hexavalent Cr (Fig. 2b). For lower concentrations, effects were strictly additive (Fig. 2a).

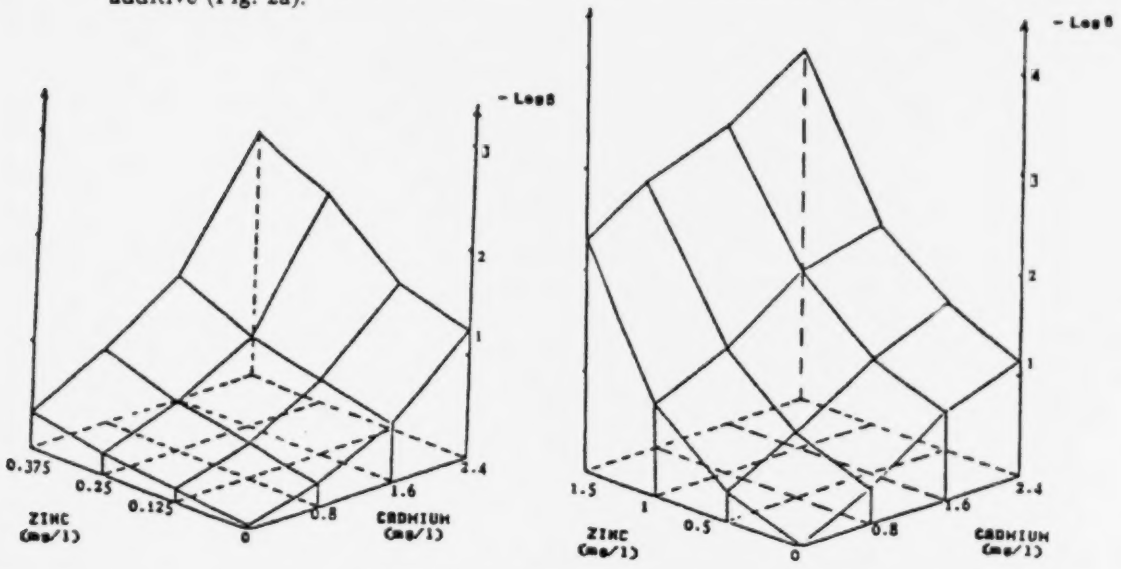

Fig. 1. Effects of Cd-Zn mixtures on the bioluminescense of *P. phosphoreum.*

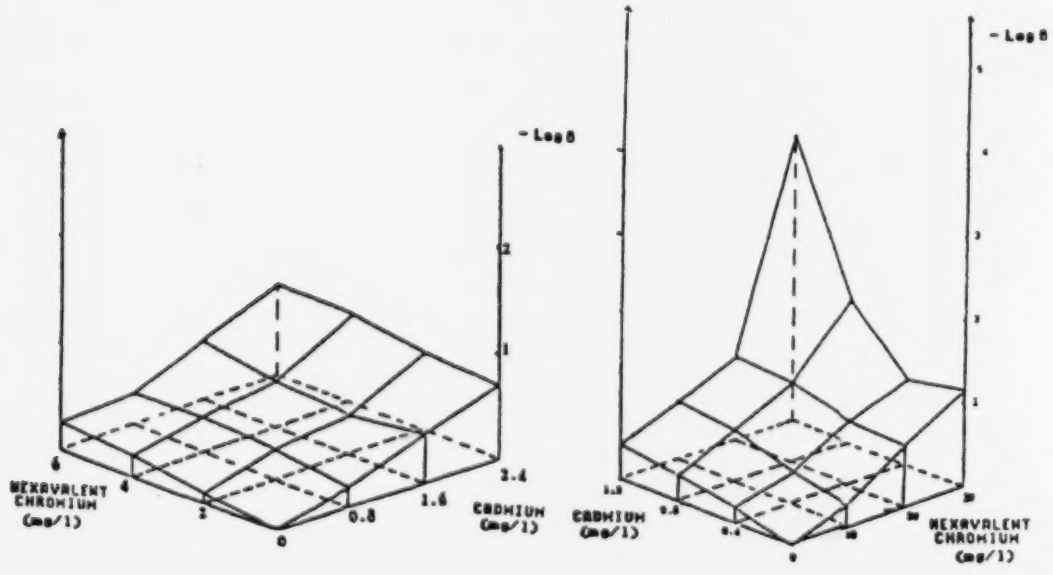

Fig. 2. Effects of hexavalent Cr-Cd mixtures on the bioluminescence of *P. phosphoreum.*

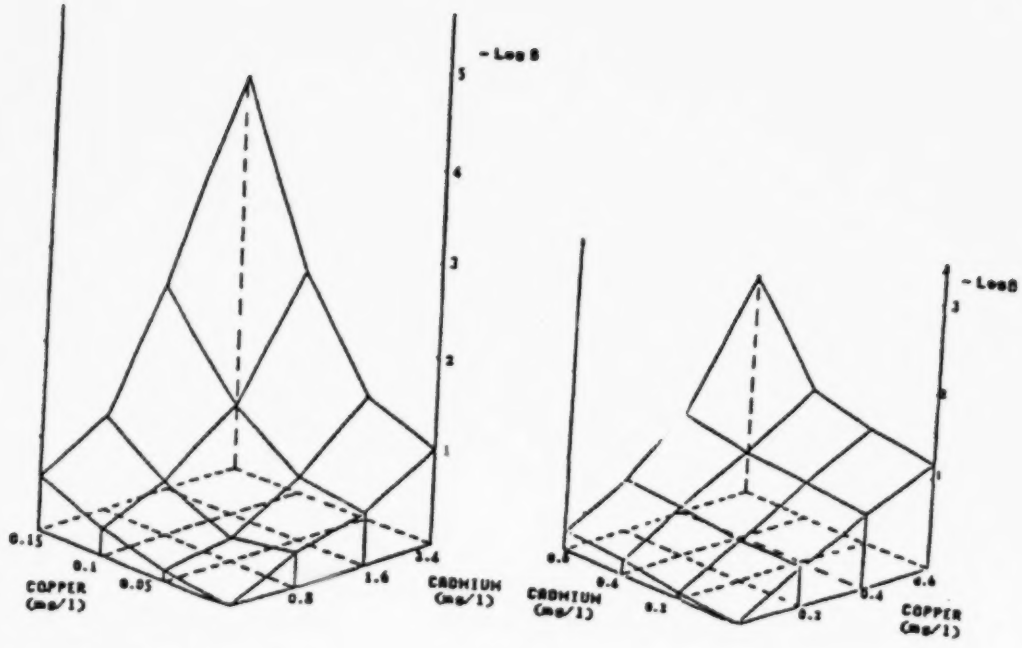

Fig. 3. Effects of Cd-Cu mixtures on the bioluminescence of *P. phosphoreum*.

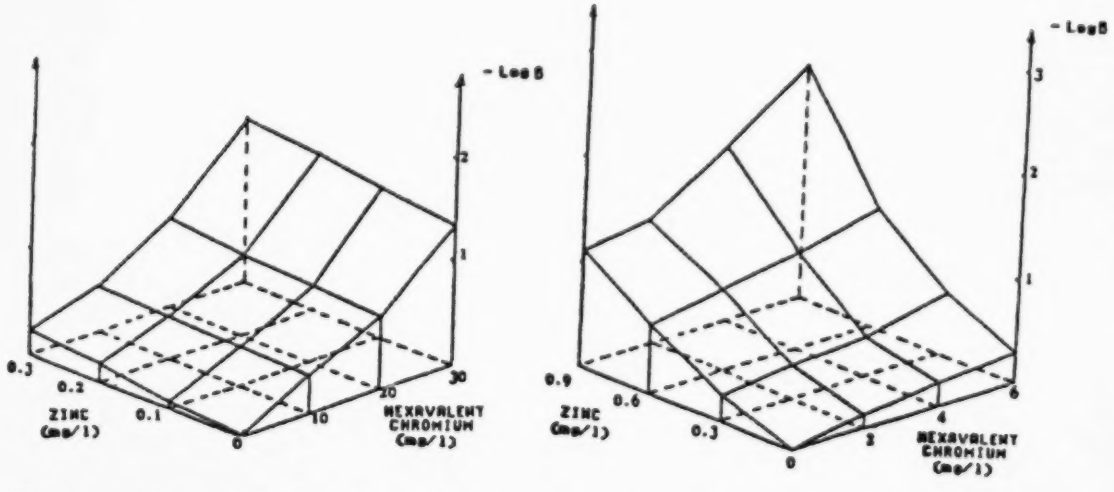

Fig. 4. Effects of hexavalent Cr-Zn mixtures on the bioluminescence of *P. phosphoreum*.

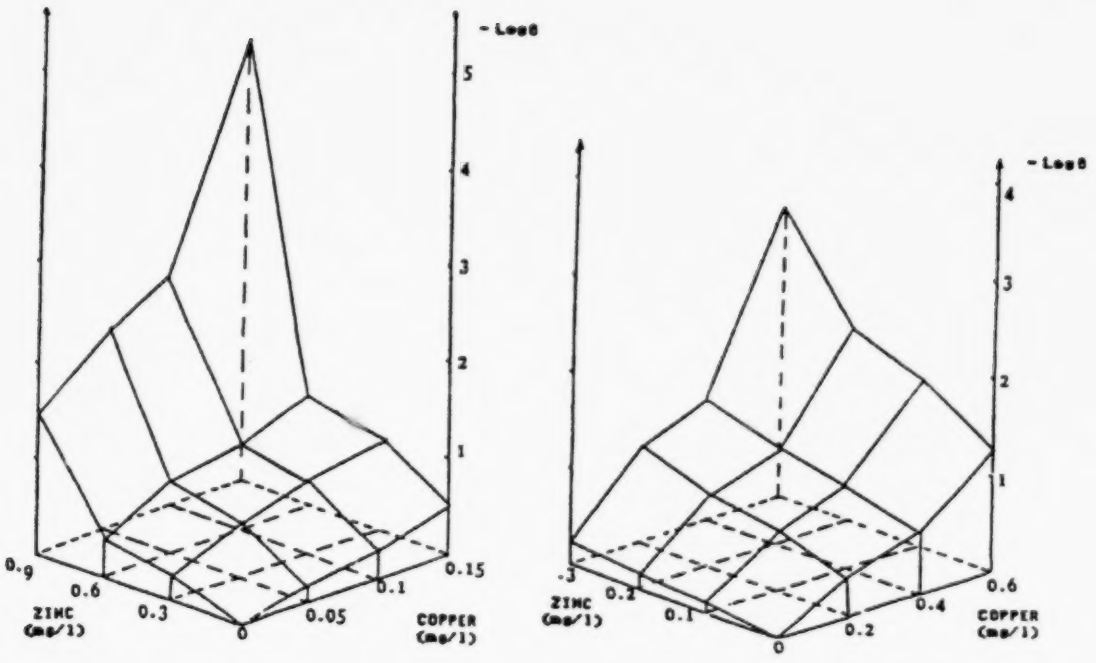

Fig. 5. Effects of Cu-Zn mixtures on the bioluminescence of *P. phosphoreum*.

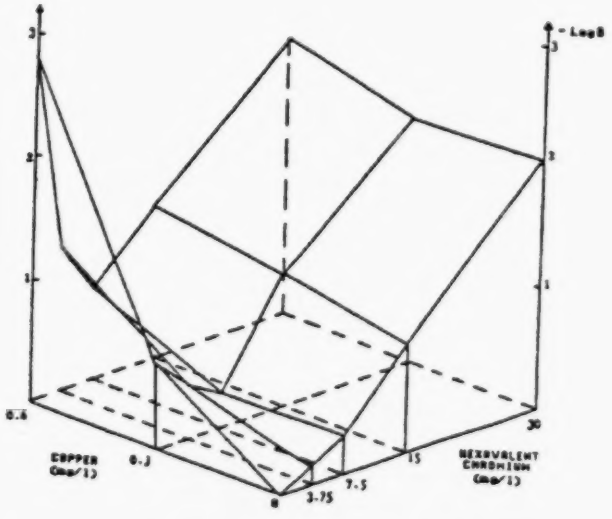

Fig. 6. Effects of hexavalent Cr-Cu mixtures on the bioluminescence of *P. phosphoreum*.

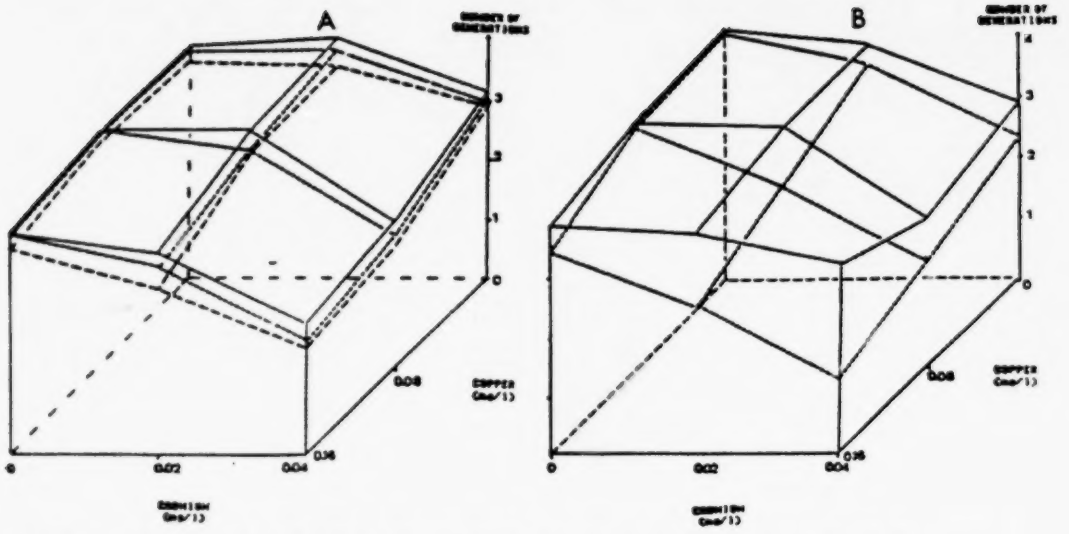

Fig. 7. (A): Effects of Cd-Cu-hexavalent Cr mixtures on the growth of *C. campylum*, (———) mixture of Cd-Cu; (·····) mixture of Cd-Cu + 3 mg/L of $K_2Cr_2O_7$; (-----) mixture of Cd-Cu + 6 mg/L of $K_2Cr_2O_7$.
(B): Effects of Cd-Cu treatment trivalent Cr mixtures on the growth of *C. campylum*, (-----) mixture of Cd-Cu; (———) mixture of Cd-Cu + 2 mg/L of trivalent Cr.

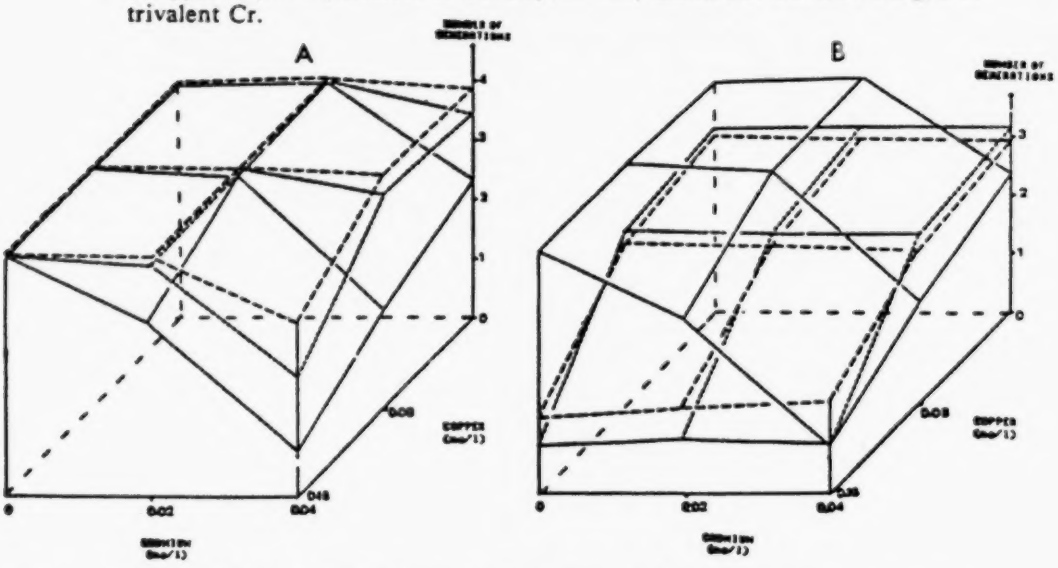

Fig. 8. Effects of Cd-Cu-Zn mixtures on the growth of *C. campylum*.
(A): (———) Mixture of Cd-Cu; (·····) mixture of Cd-Cu + 0.3 mg/L of Zn; (-----) mixture of Cd-Cu + 0.06 mg/L of Zn.
(B): (———) Mixture of Cd and Cu; (·····) mixture of Cd-Cu + 2 mg/L of Zn; (----) mixture of Cd-Cu + 4 mg/L of Zn.

Table 1. CI50 of toxicants used during study (values in mg/L).

Toxicant	CI50 Microtox	CI50 *C. campylum*
Cu^{++}	0.2±0.03	0.24±0.01
Cd^{++}	2.2±0.6	0.06±0.01
Zn^{++}	0.4±0.05	6.7±1
$K_2Cr_2O_7$	10	21.5±2.1
Cr^{+++}		>10

Cd-Cu mixtures: Synergistic effect was shown between the two metals for Cd concentrations of 1.6 and 2.4 mg/L in the presence of 0.05-0.15 mg/L Cu. At 0.8 mg/L Cd, effects of the two metals were additive (Fig. 3a). When concentrations of Cu increased (Fig. 3b), synergy was observed for lower Cd concentrations (0.6 mg/L).

Zn-bichromate mixtures: Two combinations of concentrations were studied. No interaction was observed between the two toxicants at low concentrations (Fig. 4a). At higher levels (Fig. 4b), a slight synergy appeared for Zn at 0.9 mg/L.

Zn-Cu mixtures: Figs. 5a and b clearly shows the two metals have a synergistic action on *P. phosphoreum* at all combinations of concentrations.

Cu-bichromate mixtures: The experiments showed an antagonism between the two toxicants (Fig. 6a). For concentrations of hexavalent Cr higher than 15 mg/L, the resulting toxicity was equivalent to the toxicity of Cr alone.

Effects of combined metals on *C. campylum*.

Cd-Cu-hexavalent Cr mixtures: Fig.7a shows the results of such mixtures. Only a slight synergy was observed between Cd and Cu. There was no interaction between hexavalent Cr and Cd or Cu (the three surface responses observed with the three bichromate concentrations can be obtained by accounting for the toxicity of Cr added).

Cd-Cu-trivalent Cr mixtures: Contrary to hexavalent Cr, trivalent Cr is antagonistic to both Cd and Cu, and contributes to a large decrease in the toxicity of mixtures of the other two metals.

Cd-Cu-Zn-trivalent Cr mixtures: This study was conducted with (1) non-toxic concentrations of Zn (0, 0.03 and 0.06 mg/L), and (2) toxic concentrations of Zn (0, 2 and 4 mg/l), in absence or presence of trivalent Cr. With non-toxic concentrations of Zn, a strong antagonism with Cd was observed (Fig. 8a). The synergy between Cd and Cu was confirmed. With toxic concentrations of Zn (Fig. 8b), toxicity of Cd could not be detected graphically, antagonism between Cd and Zn was very significant. No direct interaction occurred between Cu and Zn, even at high Zn concentrations, but synergy between Cd and Cu was not detected. Addition of trivalent Cr to a Cd-Cu-Zn mixture produced a spectacular decrease in toxicity (Fig. 9), this response was mainly the consequence of antagonism between trivalent Cr and Cu.

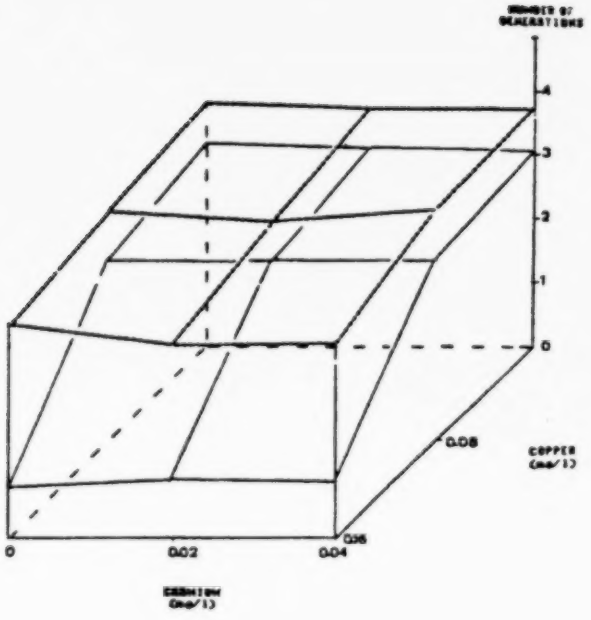

Fig. 9. Effects of Cd-Cu-Zn-trivalent Cr mixtures on the growth of *C. campylum*. (———)Mixture of Cd-Cu + 2 mg/L of Zn; (·····) mixture of Cd-Cu + 2 mg/L of Zn + 2 mg/L of trivalent Cr.

DISCUSSION

These results may be discussed (1) in terms of difference in response between the two organisms, and (2) predicting the ecotoxicological effects of electroplating wastes discharged in surface waters. Comparing biological responses, our study pointed out differences in responses between the bacterium *P. phosphoreum* and ciliate protozoan *C. campylum*. The most striking differences were in Cd and Zn, and bichromate and Cu interactions respectively. Comparison of the two models may be interesting to understand the biological mechanisms of interactions between metals. One unexpected result was a strong antagonism between trivalent Cr and Cu with *C. campylum* whereas such interaction did not occur with hexavalent Cr. A direct antagonism between divalent ions and trivalent Cr is difficult to explain in terms of biological mechanisms (competition etc.) and a precise study is required to clearly understand the origin of this interaction.

The composition of electroplating waste discharges must be considered for predicting the toxicity. Chemical analysis of a sample of such waters (Table 2) shows concentrations of hexavalent Cr is low in effluents. Trivalent Cr is more abundant but usually less than 1 ml/L in discharge. On the contrary, toxic concentrations of Cu, Cd and Zn are encountered. Significant amounts of nickel and cyanide are observed too. When the effectiveness of the treatment is not satisfactory (sample E), large amounts of toxicants are present in wastewater.

Table 2. Concentrations of the most significant toxic contaminants in five electroplating industry wastes (values in mg/L).

	Suspended matter	pH	Cn-	Cr^{+6}	Cr^{+3}	Cu	Ni	Cd	Zn
A	8	9.05	0.04	0.01	0.11	0.22	0.2	-	0.29
B	14	8.1	0.04	0.03	0.63	1.85	2.6	0.02	-
C	12	8.15	0.04	0.09	0.14	0.37	0.09	-	0.48
D	6	7.6	0.04	0.06	0.07	0.42	1.27	-	0.44
E	80	8.2	0.54	0.41	1.76	0.88	9.04	-	21.2

According to our results, estimates of toxicity for the same waste will be different in terms of the bioassay used. With the Microtox test, the toxicity measured will be in relation to Zn and Cu concentrations in the effluent and the synergy between the two metals. With the protozoan test, Cu and Cd (when present) are the most toxic components and the interaction between the two metals results in a slight increase in toxicity. Zn is, in all cases, present at a concentration for which the antagonism with Cd is present. When a high concentration of trivalent Cr is found, a decrease in toxicity can be predicted. At concentrations encountered in effluents, hexavalent Cr is not toxic and cannot interact with Cu, Cd and Zn. On the contrary, trivalent Cr can be present at concentrations which can modify the toxicity of Cu and Cd. Our study shows that neither chemical analysis or a single bioassay can give a clear estimation of the toxicological consequences of the discharge of electroplating wastes.

The antagonisms observed with the bacterium and protozoan tests cannot be considered with optimism by the industry. The decrease in toxicity related to antagonisms between metals do not correspond to a decrease in ecotoxicological risk. Synergistic effects between two metals can appear when a third, which is antagonistic, has disappeared by complexation or precipitation (the residence time of Cr in water is usually very short). So the effects of such complex effluents depend both on composition of waste at the discharge point and behaviour of components in the surface water after discharge. Water composition, particularly pH and calcium content, are very important and a study is being conducted on this subject. The role of nickel and trivalent Cr is also being examined in complementary experiments. Another factor to be considered is the presence of organic pollutants which can interact with metals. In a previous work, we have shown the presence of low concentrations of Cu can dramatically increase the toxicity of dithiocarbamates (Vasseur et al. 1986).

The procedure to develop reliable estimates of toxicity is to conduct factorial experiments in samples of different surface waters and to examine the relation between chemical composition of the waters with interactions observed in such experiments. This view is presently being practiced in our laboratories. This will allow a correct estimation of the electroplating industry wastes hazard in relation to quality of the discharge water.

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AN ENDOCRINE BASIS FOR GROWTH INHIBITION IN BROOK TROUT (*SALVELINUS FONTINALIS*) MAINTAINED IN LOW ENVIRONMENTAL PH

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ABSTRACT

Reduced growth has been observed in several fish species living in acidic lakes in central Ontario. The decrease in food supply as a result of low environmental pH has been postulated as the principal cause of diminished growth. In our previous studies, growth in brook trout maintained in the laboratory at pH 4.5 was stunted even though the fish were fed *ad libitum*, suggesting that acid stress induced physiological changes leading to reduced growth. The aim of this investigation was to study the nature of these physiological changes. Yearling brook trout were maintained at pH 4.55 with sulphuric acid and sampled frequently over a period of 2 months. At this pH, normal growth was suppressed. Morphometric analysis of the

pituitary at the electron microscope level revealed that the somatotropes and thyrotropes were atrophic and showed cytological signs of secretory inactivity, indicating that the secretion of growth hormone (GH), thyroid stimulating hormone and therefore the thyroid hormones were inhibited. However, corticotropes and interrenal cells were hypertrophic and hyperactive throughout the experiment. Plasma levels of cortisol, glucose, amino acids and proteins were significantly elevated compared with control values. The results suggest that, besides the lack of food, growth inhibition in low environmental pH is also caused by extensive endocrine changes. Reduction in GH and thyroid hormones suppress growth and anabolic activities. The elevated plasma cortisol levels also contribute to growth inhibition by stimulating catabolic processes and reducing body reserves. [Supported by NSERC Strategic Grants.]

RÉSUMÉ

On a observé une croissance réduite chez plusieurs espèces de poissons qui vivent dans des lacs acides du centre de l'Ontario. Il a été postulé que la diminution de la réserve alimentaire qui accompagnait la baisse de pH était la cause principale de cette diminution de la croissance. Lors de travaux passés, nous avons montré que la croissance d'ombles de fontaine élevés au laboratoire à pH 4.5 était réduite même si les sujets étaient nourris *ad libitum*; cela semble indiquer que le stress produit par un milieu acide entraînait des changements physiologiques conduisant à une réduction de la croissance. Le but des présents travaux était l'étude de la nature de ces changements physiologiques. Des ombles de fontaine d'un an ont été gardés à pH 4.55 dans une eau contenant de l'acide sulfurique; la population a été souvent échantillonnée pendant deux mois. Á ce pH, la croissance normale était supprimée. L'analyse morphométrique de l'épiphyse au microscope électronique a révélé que les tissus somatotropes et thyrotropes étaient atrophiés et présentaient des signes cytologiques d'inactivité sécrétoire, ce qui indique que la sécrétion de l'hormone de croissance (HC), de la thyréstimuline et donc des hormones thyroïdiennes est inhibée. Cependant, les cellules corticotropes et interrénales étaient hypertrophiées et en hyperactivité tout au long de l'expérience. La concentration en cortisol, glucose, acides aminés et protéines dans le plasma était significativement plus élevée que chez les témoins. Mise à part la question alimentaire, les résultats indiquent que l'inhibition de la croissance à bas pH est également causée par d'importantes transformations endocriennes. Le ralentissement de la production de HC et des hormones thyroïdiennes supprime la croissance et les mécanismes anaboliques. La concentration élevée en cortisol dans le plasma accentue également l'inhibition de la croissance par stimulation des mécanismes cataboliques et par réduction des tissus de réserve. (Recherche subventionnée en partie par les subventions stratégiques du CRSNG.)

PHYSIOLOGICAL DISTURBANCES IN RAINBOW TROUT DURING ACID AND ALUMINUM EXPOSURES

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ABSTRACT

Cannulated rainbow trout (*Salmo gairdneri*) were exposed to acid (pH 4.4, 4.8) and Al (0, 111 μg/L) in artificial soft water (Na=50 μeq/L; Ca=50 or 400 μeq/L). Al worsened Na^+ and Cl^- losses caused by acidity alone, and caused respiratory distress in the fish. The relative importance of these two effects depended on the experimental conditions. For example, at pH 4.8, 50 μeq/L Ca, Al-exposed fish showed moderate ion loss, and respiratory distress was indicated by low arterial 0_2, high arterial CO_2, and depressed blood pH. Al was less toxic at pH 4.4, 50 μeq/L Ca, but ion loss was greater and respiratory distress was not as severe as in the pH 4.8 experiments. Our results will be discussed in the context of gill surface chemistry.

RÉSUMÉ

Des truites arc-en-ciel canulées (*Salmo garidneri*) ont été exposées à un acide (pH 4.4, 4.8) et à l'Al (0, 111 μg/L) dans une eau douce artificiellement constituée (Na = 50 μég/L; Ca=50 ou 400 μég/L). L'aluminium a aggravé les pertes en Na^+ et Cl^- éprouvées par l'acidité seulement et a provoqué la détresse respiratoire chez les poissons. L'importance relative de ces deux effets dépendait des conditions expérimentales. Par exemple, à pH 4.8 et à 50 μeg/L Ca, les poissons exposés à l'Al mont raient une perte ionque modérée et la détresse respiratoire était révélée par une faible tension artérielle en O_2, une forte tension artérielle en CO_2 et un pH sanguin déprimé. L'aluminium s'est révélé moins toxique à pH 4.4, à 50 μéq/L Ca, mais les pertes ioniques ont été plus grandes et la détresse respiratoire moins accentuée qu'elles ne l'ont été dans les expériences faites à pH 4.8. Nos résultats seront analysés dans la perspective de la chimie à la surface des branchies.

EFFECTS OF ACID AND ALUMINUM ON SWIM BLADDER DEVELOPMENT AND YOLK ABSORPTION IN THE FATHEAD MINNOW, *PIMEPHALES PROMELAS*

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ABSTRACT

Thirty-day old fathead minnows were raised to maturity and spawning in a laboratory flow-through system using softened water at various pH and Al levels. Successful spawnings were reduced by >85% at pH 6.0, 5.5-25 ug/L Al^{+3}, and 5.5, and absent in all pH 5.2 treatments. Hatching success, larval survival, swim bladder development and yolk absorption were reduced or abnormal when spawning did occur at lower pHs.

RÉSUMÉ

Des ménés à grosse tête de trente jours ont été élevés en laboratoire jusqu'à la maturité et la fraie dans une eau adoucie en circulation permanente à différents pH et différentes concentrations d'aluminium. Le succès de la fraie a été réduit par plus de 85% à pH 6.0 et 5.5-25 μg/L Al^{+3} et à pH 5.5, et la fraie a été supprimée dans tous les développement de la vessie natatoire et l'absorption du vitellus ont été réduits ou anormaux lorsqu'il y avait fraie à has pH.

EXTENDED ABSTRACT

Thirty-day old fathead minnows were transferred to tanks containing diluted Lake Superior water at various pH and aluminum levels (pH 7.0, 7.0-40 μg/L Al^{+3}, 6.0, 5.5, 5.5-25 μg/L Al^{+3}, 5.2, 5.2-40 μg/L Al^{+3}, 5.2-70 μg/L Al^{+3}, replicate tanks for each treatment, Al values approximate). The source water for the flow-through system was maintained at a Ca^{+2} concentration of 8 mg/L, the lowest level to which the young brood stock could comfortably acclimate. Water hardness was 29.5 mg/L as $CaCO_3$. In all treatments [O_2] was at least 90% of saturation and [CO_2] less than 4.4 mg/L. The minnows were kept at a temperature of 25°C and a photoperiod of 16 h for 109 d, sufficient time for maturation and spawning to occur. A principal goal of the study was to examine larvae from successful spawnings for acid- and Al-associated abnormalities.

The minnows failed to spawn at any pH 5.2 treatment, spawned less than once per female at pH 6.0, 5.5, and 5.5-25 Al, and about 7 times per female at both pH 7.9 treatments. In general, spawning problems were more severe in the present exposures than in previous studies with this species (Mount 1973; Leino and McCormick 1984) using harder water with more Ca. Hatching success was 92-94% in control treatments, 61% at pH 6.0, 38% at pH 5.5-25 Al, and 0% at pH 5.5. Survival of larvae to 4 d was 92% in control treatments, 56% at pH 6.0, and 5.% at pH 5.5-25 Al.

Yolk in control larvae was usually absorbed by 24 h, and the fish began to feed on live brine shrimp. Yolk absorption and onset of feeding was delayed at pH 6.0 and

5.5-25 Al. By 4 days, surviving larvae at pH 6.0 had used their yolk and about 80% were feeding, whereas at pH 5.5-25 Al most larvae retained some yolk and had little or no food in their intestines.

The volume of swim bladder cavities was computed (see Downing et al. 1981) using measurements from sagittal sections of 4-d larvae. Cavity volumes of acid treated larvae were smaller than those of controls (Table 1, Figs. 1-2). The swim bladders were small with considerable interstitial fluid and underdeveloped or poor-developed lumen (Fig. 2) and appeared nonfunctional in 6 of 11 larvae exposed to pH 5.5-25 Al (Fig. 2).

Table 1. Mean volume of swim bladder cavities in 4-d old fathead minnow larvae.

pH	No. fish	Volume, mm^3 (S.D.)
7.0	11	0.039 (0.014)
6.0	9	0.024* (0.005)
5.5-25 Al	11	0.020* (0.017)

* Significantly different from pH 7.0, $p<0.05$, Bonferroni adjusted t-test (Godfrey 1985).

Control larvae which were transferred 1 d after hatching to pH 5.5-25 Al water for 14 d also had abnormal swim bladders. By 2 weeks, swim bladders of fathead minnows develop their adult configuration; an anterior and posterior chamber separated by a muscular diaphragm (Fig. 3). The transferred larvae had smaller anterior chambers (Table 2) which sometimes contained interstitial fluid in their walls (Fig. 4).

Table 2. Mean volume of swim bladder cavities in 15-d old fathead minnow larvae.

pH	No. fish	Volume, mm^3 (S.D.)	
		Anterior chamber	Posterior chamber
7.0	5	0.052 (0.016)	0.014 (0.05)
5.5-25 Al*	5	0.013**(0.007)	0.11 (0.02)

* Larvae transferred from pH 7.0 to pH 5.5-25 Al at 1 d.
** Significantly different from pH 7.0, $p<0.01$, t-test.

The present experiments suggest that in soft water, fathead minnow spawning success and larval survival, yolk utilization, and swim bladder volume are affected at pH 6.0. Larval feeding, yolk absorption, and swim bladder development are markedly inhibited at pH 5.5-25 Al (although the Al may offer some protection at this pH since at pH 5.5-0 Al and below adults were unable to spawn or eggs were unable to survive). Retarded yolk absorption and swim bladder development seem to indicate a direct effect of low pH on metabolism during the critical period of transition to exogenous feeding, in addition to spawning problems, could contribute to recruitment failure.

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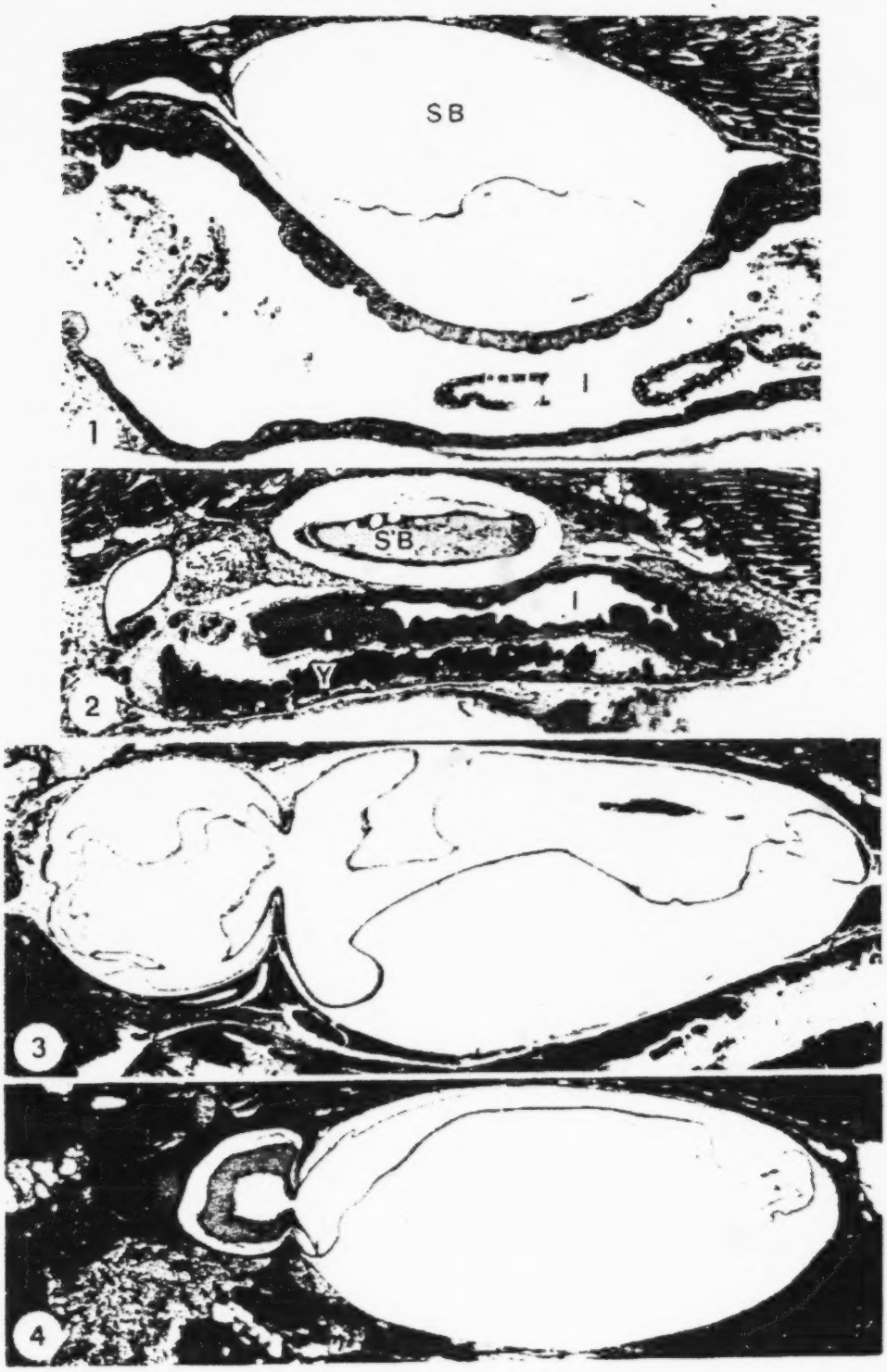
PHOTOMICROGRAPHS

Fig. 1. Sagittal section through control (pH 7.0) 4-d old fathead minnow larva showing inflated swim bladder (SB) and expanded intestine (I) containing food debris. No yolk is present. x120.

Fig. 2. Section through 4-d larva, pH 5.5-25Al, showing unexpanded, fluid-filled swim bladder, empty intestinal lumen, and yolk remnants (Y). x120.

Fig. 3. Swim bladder of 15-d fry, pH 7.0, showing anterior (left) and posterior chambers. x80.

Fig. 4. Swim bladder of 15-d fry, pH 5.5-25Al, showing abnormally small anterior chamber with fluid in its wall. x80.


SB
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SB
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BIOESSAIS DE DIFFERENCIATION DES EFFETS DE L'ALUMINIUM CHEZ LES POISSONS EN RELATION AVEC LES PRECIPITATIONS ACIDES

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RÉSUMÉ

Al issu des sols infiltrés par les précipitations acides exerce un effet toxique chez les poissons des eaux modéremment acides (pH 5.5). Cette action toxique survient à plusieurs niveaux histophysiologiques chez *Salvelinus fontinalis*.

Dans les branchies, les lamelles secondaires montrent un rétrécissement de leur sommet et un élargissement de leur base; ceci est surtout dû a l' acidité. Al se bioaccumule assez rapidement dans les branchies: il y est non seulement absorbé mais aussi incorporé, notamment aux membranes des cellules épithéliales d'échange. En réponse à cette double agression toxique synergique, il y a une augmentation de la synthèse des ARN et des protéines dans les branchies: cette augmentation s'observe à pH 5.5 et est amplifiée avec l' addition de 300 ug Al/L (bien que seulement 25% d'Al soit biodisponible surtout sous forme de $Al(OH)^{++}$ à ce pH).

A l'intérieur de l'organisme, la teneur plasmatique de Na^+ diminue ce qui entraîne une augmentation des hormones urotensines (II principalement) et une hypertrophie de leurs cellules neurosécrétrices dans l'urophyse; parallelment, la concentration de la vasotocine pituitaire s'accroît. Ces phénomènes iono-osmorégulateurs sont davantage reliés à l'acidité qu'à Al. Ce dernier agit toutefois dans l'organisme: il y provoque entre autres une hausse de la synthèse des ARN et des protéines dans le foie, lesquelles protéines additionnelles contiennent une part appréciable Al et équivaudraient à des metallothionéines.

L'ensemble des résultats indique que la toxicité de Al se conjugue avec celle de l'acidité chez les poissons. De plus, le métal s'y bioaccumule dans les branchies d'abord et l'organisme ensuite et y induit une surproduction de protéines en réponse à l'agression.

Globalement, les truites se révélent plus sensibles à Al qu'à l'acidité modérée comme le montrent des bioessais de préférence. La toxicité de Al s'y exerce selon des mécanismes particuliers supplémentaires à ceux de l'acidité.

ABSTRACT

The Al from soils suffering from acid precipitation infiltration has a toxic effect on fish in moderately acidic waters (pH 5.5). The toxic effect can be seen at several histo-physiological levels in *Salvelinus fontinalis*.

The secondary lamellae of the gills become narrower at the top and wider at the base, mainly as a result of the acidity. The Al accumulates quite rapidly in the gills: it is not only absorbed, but also incorporated, notably in the membranes of replacement epithelial cells. In response to these two actions with its synergistic toxic

effects, there are increases in the syntheses of RNA and proteins in the gills. This is seen at pH 5.5, and increases with the addition of 300 μg Al/L (though at this pH, only 25% of the Al is available, mainly in the form of $Al(OH)^{++}$).

Within the organism, Na^+ content of the plasma decreases, causing an increase in urotensin hormones (mainly II) as well as hypertrophy of the corresponding neurosecretory cells in the urophysis. At the same time, concentration of pituitary vasotocin increases. These ionic-osmoregulatory processes are related more to acidity than to Al. The Al nevertheless does have effects within the organism: amongst other things, it causes increases in syntheses of RNA and proteins in the liver. These additional proteins contain an appreciable quantity of Al, so that they are equivalent to metallothioneins.

The results as a whole indicate that in fish, the toxic effects of Al are added to the effects of acidity. Also, metal accumulates first in the gills, then in the organism as a whole, where it causes an overproduction of proteins.

Overall, the fish examined were more sensitive to Al then to moderate acidity, as shown by preferential bioassays. Al has its toxic effect through specific mechanisms that operate in addition to those through which acidity acts.

ZINC INFLUX IN THE RAINBOW TROUT AND THE EFFECTS OF ACUTE CHANGES IN WATERBORNE [CA]

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ABSTRACT

A multifactorial diet/waterborne exposure indicated that water could be an important source of Zn to hard water-acclimated ([Ca]=36 mg/L) rainbow trout (*Salmo gairdneri*). To establish actual flux rates, and possible routes of uptake, a kinetic method was devised whereby cannulated trout were "calibrated" by the infusion of ^{65}Zn. Steady state activity in the plasma occurred after 24-36 h, and was a linear function of influx rate (r=0.88). Uncannulated trout were then exposed to ^{65}Zn and a terminal blood sample taken. Influx rate was interpolated from the relationship. When influx rate was studied at various [Zn], influx was not a simple linear function of concentration, but saturated rapidly. Acute changes in waterborne [Ca] (1-194 mg/L) altered Zn influx in two different ways. Both increases and decreases influenced the saturable component in a classical competitive manner (changes in K_m with little change in V_{max}), whereas removal of Ca alone opened an unsaturable pathway. These data suggest that the saturable phenomenon arose from Zn traversing the gill in a manner similar to the recently elucidated mechanism for branchial Ca uptake. The linear phenomenon in contrast, resulted from the opening of non-specific paracellular leak pathways.

RÉSUMÉ

Une exposition à plusieurs facteurs par l'eau et par l'alimentation a indiqué que l'eau pouvait constituer une source importante de Zn pour les truites arc-en-ciel (*Salmo gairdneri*) acclimatées à l'eau dure ([Ca=36 mg/L). Pour calculer le flux réel et les voies possibles d'assimilation, une méthode cynétique a été mise au point: des truites canulées ont été "calibrées" par infusion de ^{65}Zn. L'équilibre dans le plasma a été atteint après 24-36 h et a varié linéairement en fonction de l'influx ($r = 0.88$). Des truites non canulées ont ensuite été exposées au ^{65}Zn et on ensuite été saignées à blanc. L'influx a été calculé par interpolation à partir de cette relation. Quand il était étudié à différentes [Zn], l'influx ne variait pas linéairement avec la concentration, mais il y avait une saturation rapide. Des changements aigus de [Ca] dans l'eau (1-194 mg/L) modifiaient l'influx de Zn de deux façons différentes. Une augmentation comme une diminution agissait sur la valeur à saturation selon les cas classiques de compétition (variation de K_m avec peu de variation de V_{max}), alors que la suppression du Ca seulement donnait lieu à une insaturation. Ces résultats indiquent que le phénomène de saturation était issu du passage du Zn par les branchies selon un mécanisme semblable à celui récemment trouvé pour expliquer l'absorption de Ca par les branchies. Par contraste, la linéarité du rapport résultait de la création de voies paracellulaires non spécifiques.

ACCUMULATION AND ELIMINATION OF INORGANIC AND ORGANIC FORMS OF DIETARY SELENIUM IN RAINBOW TROUT, *SALMO GAIRDNERI*

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ABSTRACT

Selenium is relevant to aquatic biota, both as a pollutant and as a potential ameliorative agent for mercury contaminated aquatic ecosystems. Dietary sources of selenium are known to be important for fish.

This experiment examined the dietary uptake of organic (^{75}Se-cystine and ^{75}Se-methionine) and inorganic ^{75}Se-selenate and ^{75}Se-selenite) forms of selenium by juvenile rainbow trout (*Salmo gairdneri*). An uptake period of 60 d was followed by a 14 d depuration period. The uptake ratio constant (K_1), clearance rate constant (K_2), and half-life ($T_{0.5}$) were respectively: 0.0051 and 0.0471 per d, and 14.7 d for ^{75}Se-ate; 0.0089 and 0.0635 per d, and 10.9 d for ^{75}Se-cys; 0.0091 and 0.0442 per d, and 15.6 d for ^{75}Se-ite; and 0.0063 and 0.0317 per d, and 21.8 d for ^{75}Se-met. Biomagnification factors (Km) for the treatments were (in decreasing order): 0.205 for ^{75}Se-ite; 0.199 for ^{75}Se-met; 0.141 for ^{75}Se-cys; and 0.109 for ^{75}Se-ate.

It is concluded that selenite is accumulated by rainbow trout more efficiently than seleno-animo acids, and that selenate is a poor dietary source of selenium for trout. Biomagnification does not occur in rainbow trout from any of the food sources used.

RÉSUMÉ

Le sélénium est un élément important dans les biotes aquatiques tant comme polluant que comme agent potentiel d'amélioration des écosystèmes aquatiques contaminés par le mercure. C'est un fait connu que les sources alimentaire de sélénium ont de l'importance chez les poissons.

Cette expérience dont il est question ici visait à l'examen de l'absorption par les aliments de formes organiques (^{75}Sé-cystine et ^{75}Sé-méthionine) et de formes inorganiques (^{75}Sé-séléniate et de ^{75}Sé-sélénite) chez des truites arc-en-ciel juveniles (*Salmo gairdneri*). Une période d'absorption de 60 d a été suivie d'une période dépuration de 14 d. La constante du taux d'absorption (K_1), la constante du taux de clearance (K_2) ainsi que la demi-vie ($T_{0.5}$) ont été les suivantes: 0.0051 et 0.0471 par d, et ainsi que 14.7 d pour le ^{75}Sé-ate; 0.0089 et 0.0635 par d, ainsi que 10.9 d pour le ^{75}Sé-cys; 0.0091 et 0.0442 par d, ainsi que 15.6 d pour le ^{75}Sé-ite; enfin, 0.0063 et 0.0317 par d, ainsi que 21.8 d pour la ^{75}Sé-mét. Les facteurs de biomagnification (Km) des truitements ont été (dans l'ordre décroissant): 0.205 pour le ^{75}Sé-ite, 0.199 pour la ^{75}Sé-mét, 0.141 pour la ^{75}Sé-cys et 0.109 pour le ^{75}Sé-ate.

Il est conclu que le sélénite est retenu plus efficacement que les acides sélénoaminés par la truite arc-en ciel et que le sélénate constitue une mauvaise source alimentaire de sélénium pour la truite. Aucune des sources alimentaires testées ici n'a conduit à une bioamplification chez la truite arc-en-ciel.

THE TOXICITY OF POTASH BRINE TO ATLANTIC SALMON (*SALMO SALAR*) EGGS, ALEVINS AND FRY

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ABSTRACT

Recent spills of brine from potash mines into an Atlantic salmon stream have raised concerns about effects on resident fish. Potash 7-d LC50s measured by static bioassay had conductivities of 20,500-26,300 (92.0-118.0 mM K), 10,740 (48.2 mM K) and 3,600 (16.0 mM K) μS for eggs, alevins and fry, respectively. The ionic composition of saturated potash brine was 2129, 1827, 6.61, 4113 and 0.617 nM for Na, K, Ca, Cl and Mg, respectively. The main toxic element in the brine was ascertained to be K, the 7-d LC50 being 49.4 mM K for alevins, therefore this seems to account for the total brine toxicity. Potash brine affected the water hardening process of newly fertilized salmon eggs reducing water uptake significantly ($p<0.005$). Blastodisc growth was also retarded at the lowest concentration tested (395 μS, 1.8 mM K). Considering the most sensitive developmental stages tested, early cleavage and newly feeding fry, an increase in river conductivity due to potash brine inflow above 360 μS would have deleterious effects on Atlantic salmon survival.

RÉSUMÉ

Récemment des fuites de saumûre, provenant des mines de potasse, dans des ruisseau où habitent du saumon de l'Atlantique, a provoqué de l'inquiétude à propos des effets possible sur ces poissons. La potasse 7-d LC50s, mesuré avec des essais biologiques statique, avait des conductivités de 20,500-26,300 (92.0-118.0 nM K), 10,740 (48.2 mM K) et 3,600 (16.0 mM K) μS pour les oeufs, les alevins et les frétins, respectivement. La composition ionique de la saumûre saturée de potasse était de 2129, 1827, 6.61, 4113 et 0.617 mM pour le Na, K, Ca, Cl et Mg, respectivement. Puisque 7-d LC50 était 49.4 mM K pour les alevins, K fut établit comme étant l'élément toxique principal de la saumûre et conséquemment semblait compté pour la toxicité totale de la saumûre. La saumure de potasse avait un effet sur le processus du durcissement de l'eau dans les oeufs de saumon récemment fertilisés, réduissant ainsi significantivement ($p<0.005$) l'absorption de l'eau. La croissance "bastodisc" fut aussi retardée àla concentration la plus basse analyseé (395 μS, 1.8 mM K). Si on considére que le stade de développement entre le début du clivage jusqu'aux jeunes frétins commencant à se nourrir est le plus susceptible, un accroissement de la conductivité dans les riviéres causé par de la saumûre de potasse dépassant 360 μS pourrait avoir effet délétère sur la survie du saumon de l'Atlantique.

THE EFFECTS OF WATER TEMPERATURE ON CHRONIC TOXICITY OF SODIUM ARSENATE TO RAINBOW TROUT (*SALMO GAIRDNERI*)

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ABSTRACT

Acute bioassays indicate that trout acclimated to 5°C were more tolerant to sodium arsenate ($Na_2HAsO_4.7H_2O$) than acclimated to 15°C. The 144 h LC50's were 115 mg/L and 58 mg/L respectively. However, during a 11 wk chronic study at concentrations of 0.15 and 0.30 of the LC50, the 15°C trout were less affected than the 5°C trout. At the highest concentration (0.30 of the LC50) up to 50% mortality was observed at 5°C while less than 15% mortality occurred at 15°C. Mortalities occurred daily throughout the study at 0.30 of the LC50 (5°C) yet inhibition of growth by 11 wk was minimal (not significant) and effects on liver glycogen were not evident. The results show that tolerance is increased at 5°C during chronic exposure.

Whole body uptake kinetics indicate that arsenate is not bioconcentrated and that mortalities are related to a constant body burden. Tolerance changes can be explained by alteration of metabolic rate and time to reach a critical body burden. Further analysis on biomethylation and biotransformation of arsenate will be discussed with reference to the chronic mortality and the ability of the trout to metabolize arsenate at two water temperatures.

RÉSUMÉ

Des essais biologiques visant à l'étude de la létalité aigue indiquent que la truite acclimatée à une eau de 5°C tolère mieux l'arséniate de sodium ($Na_2HAsO_4.7H_2O$) que les truites acclimatées à une eau de 15°C. Les CL50 de 144 h étaient de 115 mg/L et 58 mg/L, respectivement. Cependant, lors d'une étude de létalité chronique de 12 semaines à des concentrations de 0.15 et 0.30 de la CL50, les truites acclimatées à l'eau de 15°C étaient en meilleure santé que celles acclimatées à l'eau de 5°C. A la plus forte concentration (0.30 de la CL50), une mortalité atteignant 50% a été observée chez le sujets acclimatés à l'eau de 5°C alors que cette mortalité était de moins de 15 % chez les sujets acclimatés à l'eau de 15°C. Il y a eu des mortalités tous les jours dans les tests à 0.30 de la CL50 (5°C), mais pourtant l'inhibition de la croissance au bout de 12 semaines était minimale (non significative) et les effets sur le glycogène du foie n'étaient pas apparents. Les résultats montrent que la tolérance est accrue à 5°C durant une exposition chronique.

La cynétique de l'absorption par l'organisme indique que l'arséniate n'est pas bioaccumulé et les cas de mortalité sont en rapport avec une charge constante dans l'organisme. Les variations de tolérance peuvent être expliquées par une altération du métabolisme et le temps mis pour atteindre la charge critique dans un organisme. L'analyse de la biométhylation et de la biotransformation de l'arséniate sera examinée dans la perspective de la mortalité chronique et de la capacité des truites à métaboliser l'arséniate au deux températures de l'eau.

EXTENDED ABSTRACT

Arsenic is widespread throughout the aquatic environment and can exist in many forms. There are four oxidation states of arsenic with arsenate (pentavalent) being the most stable (NRCC, 1978). The major sources of arsenic discharge into the environment are metal smelting, mining and fossil fuel combustion. As a result of arsenic's widespread occurrence, its toxicity can be influenced by a number of modifying factors, most notably temperature.

The purpose of this study is to investigate the effects of water temperature on the toxicity of sodium arsenate ($Na_2HAsO_4.7H_2$)) to rainbow trout (*Salmo gairdneri*) over a 11 wk exposure. During this exposure whole body arsenic burden (uptake), growth, and liver and muscle glycogen were analyzed.

Initial acute (144 h LC50) bioassays indicated that trout acclimated to 5°C were twice as tolerant to arsenate than trout acclimated to 15°C. The 144 h LC50 at 5°C was 115 mg/L while at 15°C it was 58 mg/L. However, during 11 wk chronic study, the 15°C trout were less affected than the 5°C trout. The nominal concentrations tested were 1.5 mg/L, 9 mg/L and 18 mg/L at 15°C while 5°C nominal concentrations were 1.5 mg/L, 18 mg/L and 36 mg/L. The latter two concentrations at each temperature represented 0.15 and 0.30 of the 144 h LC50 for that particular temperature. At the highest concentration (0.30 of the LC50), up to 60% mortality was observed at 5°C while less than 15% mortality occurred at 15°C. At 5°C, the mortalities occurred daily throughout the study at 36 ml/L (0.30 of the LC50) while inhibition of growth during the 11 wk was minimal (Fig. 1). While this reduction in growth (day 77) is significant ($p<0.05$), it is not very meaningful since it does not become evident until after 4 wk of exposure and numerous deaths. Exposure to lower concentrations of arsenate (1.5 and 18 mg/L) show no effect on weight growth at 5°C (Fig. 1). No

reduction in wet weight growth occurred during the 11 wk exposure at 15°C (Fig. 2), yet at the highest concentration (18 mg/L) up to 15% mortality occurred. Work by Rankin (1987) found that this reduction in growth is attributable to loss of appetite and not poor energy conversion efficiency.

The major parameter measured in this study was arsenic whole body burden. This was measured as total arsenic by neutron activation analysis at McMaster University, Hamilton. Results at both temperatures (Fig. 3) shows a good dose-response relationship, arsenic is easily taken up by fish, and its ability to bioconcentrate is very low (< 1μg/g). Since the control body burdens for Fig. 3 were not significantly different from the low arsenate exposure treatments they were omitted from the diagram. When the 15°C trout are exposed to arsenate, equilibrium was not attained until 28 d post exposure and by 77 d the trout were eliminating more arsenic than they were accumulating. The results at 5°C suggest a similar finding, however, there was no reduction in body burden occurring within the 77 d exposure (Fig. 3). Exposure to each temperatures 0.3 of the LC50 (18 mg/L-15°C; 36 mg/L-5°C) resulted in very similar uptake patterns (Fig. 3) and body burdens reached levels of 2.5-3.5 μg arsenic/g wet weight. While these body burdens (total arsenic) may be similar, mortality rate at 5°C was much greater. This would indicate either a greater ability to withstand these body burdens at 15°C or a more efficient mechanism in detoxifying arsenate to less toxic organic forms. It appears most likely that a better biotransformation or detoxification mechanism is responsible for greater survival at 15°C during chronic exposure. Work by McGeachy et al. (1987) had found a critical arsenic body burden which cause death or loss of equilibrium range from 6-9 μg arsenic/g wet weight for trout exposed to lethal concentrations of arsenate at 5°C or 15°C. Thus trout held at 15°C may not have the ability to withstand greater arsenic body burdens.

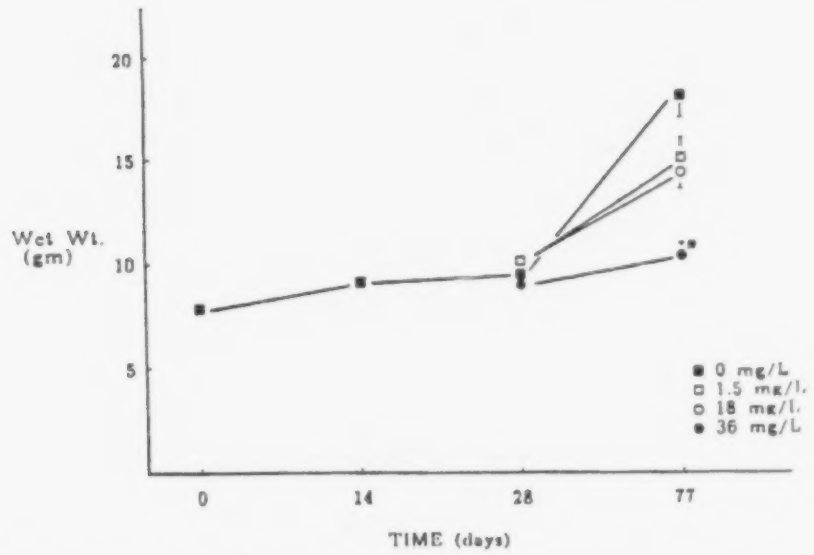

Fig. 1. Mean wet weight (growth) of rainbow trout exposed to arsenate. Error bars = SEM.

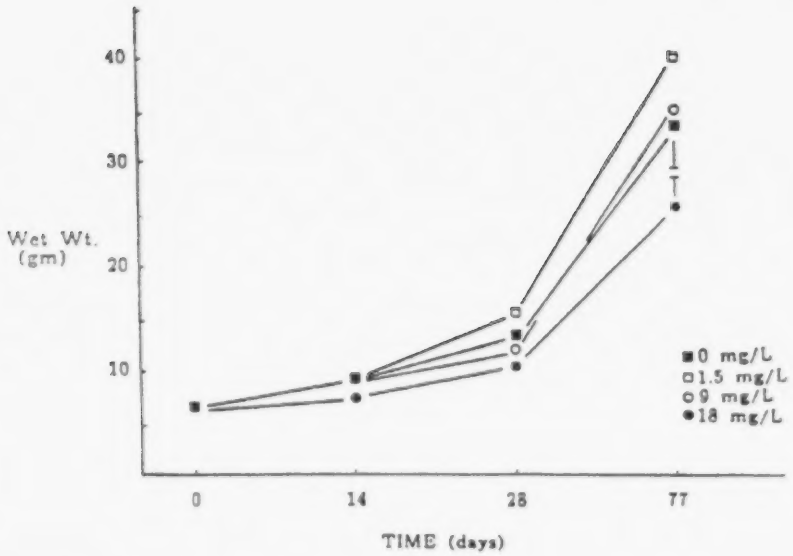

Fig. 2. Mean wet weight (growth) of rainbow trout exposed to arsenate. Error bars = SEM.

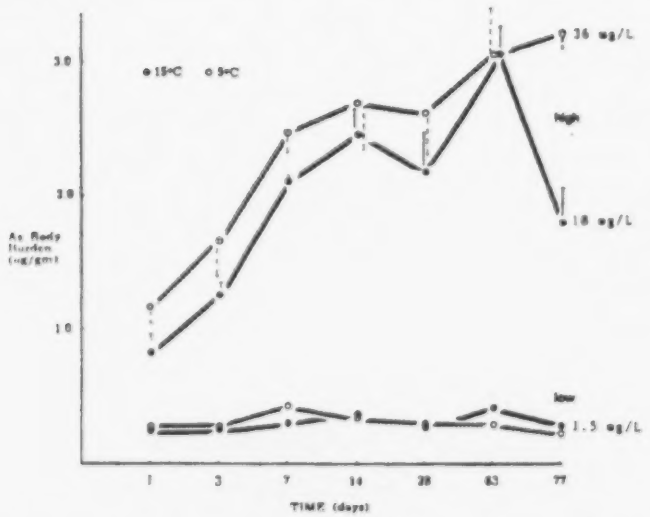

Fig. 3. Whole body burden (total arsenic) of rainbow trout exposed to arsenate. Error bars = SEM.

There were no major effects of arsenate on liver glycogen content. Mean liver glycogen content after 28 d for control fish was 32 mg/g while glycogen content for exposed treatments ranged from 28-36 mg/g. By 77 d, the effects of arsenate exposure on liver glycogen began to occur in the high treatment (5°C) as glycogen levels were reduced to 18 mg/g. However, as was observed with growth, the effects on liver glycogen come long after the high rates of mortality have occurred.

In summary, the relationship for the effects of temperature on arsenate toxicity to rainbow trout reverses from acute exposure to chronic exposure. A higher water temperature increases the sensitivity of trout to arsenate during an acute exposure while during a chronic exposure the higher water temperatures induce greater resistance and survivability. It was also found that during chronic arsenate exposure, there is a fine line between lethal and sublethal effects with regard to growth and liver glycogen. Also, arsenic body burdens are the same at both temperatures but mortalities were significantly greater for the 5°C trout tested. It is postulated that the 15°C trout are better able to detoxify the arsenate to less toxic organic forms. More research is required in this area.

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THE EFFECT OF NICKEL PRE-EXPOSURE ON THE LETHAL TOLERANCES OF THE ZEBRAFISH (*BRACHYDANIO RERIO*)

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ABSTRACT

The purpose of this study was to investigate acclimation-induced changes in tolerance of fish through pre-exposure to Ni. Pre-exposure of zebrafish (*Brachydanio rerio*) to waterborne Ni at concentrations equal to 0.03, 0.06, and 0.28 of the 144 h LC50 for 7 d lead to a decrease in tolerance (i.e. sensitization) to lethal levels of Ni compared to controls. A similar trend of sensitization was seen when fish were challenged with lethal Cu levels following a 7 d acclimation to Ni at 0.08 of the 144 h LC50. This response was not diminished by extending length of the pre-exposure period to 14 d.

Two hypotheses concerning the sensitization caused by Ni acclimation are currently being explored. The first being that Ni pre-exposure modifies subsequent uptake of either Ni or Cu resulting in increased bioconcentration of these metals during the acute lethality phase. The second hypothesis proposes that Ni may depurate slowly from the body. The body levels of Ni reached during the pre-exposure period may therefore be either additive or interactive with those of Ni or Cu during the acute lethality test phase.

The contrast between the tolerance modifications which arise through Ni acclimation and those which arise through certain other metals such as Cu, Cd, and Ag will be discussed.

RÉSUMÉ

La présente étude avait pour objectif l'examen de variations de tolérance induites par une acclimatation de poissons par exposition préalable au Ni. L'exposition préalable de (*Brachydanio rerio*) à du Ni contenu dans l'eau en concentration égales à 0.03, 0.06 et 0.28 de la CL 50 144 h pendant 7 jours a conduit à une diminution de la tolérance c.-à-d. une sensibilisation) à des concentrations létales de Ni par comparison aux témoins. Une tendance semblable à la sensibilisation a été observée quande des poissons ont été avec du Cu en concentration létale après une acclimatation de 7 jours au Ni à 0.08 de la CL50 144 h. Cette réaction n'a pas été diminuée par le prolongement jusqu'à 14 jours de la durée de la période d'exposition préalable.

Deux hypothèses concernant la sensibilisation obtenue par acclimatation au Ni sont actuellement examinée. Selon la première, l'exposition préalable au Ni modifierait l'absorption subséquente de Ni ou du Cu, ce qui se traduirait par une bioconcentration accrue de ces métaux durant la phase du test de létalité aiguë. Selon la deuxième hypothèse, le Ni pourrait être dépuré lentement de l'organisme. Les concentrations atteintes en Ni dans les organismes durant la période de l'exposition préalable pourraient donc s'additionner ou avoir un effet synergique avec les concentrations de Ni ou de Cu durant la phase du test de létalité aiguë.

Le contraste entre les variations observées de tolérance issues de l'acclimatation au Ni et de l'acclimation à certains autres métaux comme le Cu, le Cd et Ag, sera analysé.

SUSCEPTIBILITY OF *DAPHNIA MAGNA*, *DAPHNIA PULEX*, *MACROCYCLOPS FUSCUS* AND *DIAPTOMUS* SP. TO METHOPRENE UNDER ACUTE AND CHRONIC EXPOSURES

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ABSTRACT

Methoprene is a juvenile hormone analogue used for mosquito control. It acts by impairing insect metamorphosis. The SR-10 formulation consists of microencapsulated methoprene at 10% AI. This work was undertaken to determine the susceptibility of nontarget species found in the same habitat as mosquito larvae.

Using the SR-10 formulation, we determined in toxicity tests performed under laboratory conditions: (1) an LC50 (72 h) of 20 μg/L (AI) for young *Daphnia*; (2) under chronic exposure survival of organisms was affected at 10 μg/L and an effect on reproduction was detected at 0.5 μg/L; (3) survival and metamorphosis of copepods was not affected at exposures up to 100 μg/L.

These results demonstrate the adequacy of *Daphnia* as test organisms to study effects of methoprene on crustacean species and the importance of considering sublethal effects when assessing the effects of environmental pollutants on aquatic organisms.

RÉSUMÉ

Le méthoprène est un analogue d'une hormone juvénile utilisé pour la lutte contre les moustiques. Il agit en interférant avec la métamorphose des insectes. La formulation SR-10 consiste en du méthoprène microencapsulé à 10 % d'IA. La présente étude a pour objectif de déterminer la susceptibilité d'espèces non visées qui sont trouvées dans le même habitat que les larves des moustiques.

Ave la formulation SR-10, nous avons déterminé ceci en laboratoire: 1) une CL50 (72 h) de 20 μg/L (IA) chez les jeunes *Daphnia*; 2) avec une exposition chronique, la survie des organismes était modifiée à 10μg/L et un effet sur la reproduction était détecté à 0.5 μg/L; 3) la survie et la métamorphose des copépodes sont demeurées intactes à des expositions atteignant 100 μg/L.

Ces résultats montret que *Daphnia* est un organisme qui se prête bien à l'étude des effets du méthoprène sur les crustacés et indiquent aussi l'importance de prendre en considération les effets sublétaux lors d'une évaluation des sur les organismes aquatiques des polluants appliqués dans le milieu.

IMPACT OF AGRICULTURAL PYRETHROID PESTICIDES ON AQUATIC FAUNA

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ABSTRACT

The synthetic pyrethroid pesticides are increasing in popularity as agricultural insect control agents. While these products are extremely effective against target pest species, their potential to cause adverse effects on non-target species, particularly aquatic fauna, is of concern to regulatory agencies. The registered use pattern for pyrethroid pesticides requires that, when the products are applied by air, a 100 m setback is required from watercourses. A study was undertaken to determine the effectiveness of watercourse setbacks in preventing unacceptable aquatic impacts. The study contrasted deltamethrin deposit and biotic effects on two agricultural ponds, one protected by a 100 m setback and one directly oversprayed. Mean measured pesticide deposit and mean measured surface water concentrations in the setback pond was reduced by approximately 1/145 and 1/450 respectively compared to the oversprayed pond. The total numbers of benthic invertebrates on artificial substrates in the oversprayed pond declined throughout the sampling period. No decline in total numbers of invertebrates was observed in the setback pond. Caged invertebrates in the oversprayed ponds suffered varying degrees of mortality. The most heavily impacted were water boatmen, chironomids and mites. Caged invertebrates in the setback pond suffered little or no mortality. Thirty percent of caged brook trout in the oversprayed pond died by the time the study was terminated, while no trout mortalities occurred in the setback pond. These results indicate 100 m setbacks are necessary in protecting watercourses from aerial applications of deltamethrin.

RÉSUMÉ

Les pesticides pyréthroïdes synthétiques gagnent en popularité comme moyen de lutte contre les insectes nuisibles aux récoltes. Ces produits sont extrêmement efficaces contre les espèces nuisibles visées, mais la nocivité potentielle pour des espèces qui ne sont pas visées, particulièrement la faune aquatique inquiète les agences de réglementation. La méthode d'tilisation enregistrée des pesticides pyréthroïdes prévoit que lorsque ces produits sont appliqués du haut des airs, il faut ménager une bande de 100 m de largeur le long des voies d'eau. Une étude a été entreprise afin de déterminer l'efficacité de ces zones tampons pour la protection des organismes aquatiques. L'étude visait à comparer le dépôt de deltaméthrine et ses effets biologiques sur deux bassins en zone agricole, l'un protégé par une zone tampon de 100 m, l'autre arrosé avec l'insecticide. Le dépôt mesuré moyen de pesticide et la concentration mesurée moyenne à la surface de l'eau dans le bassin protégé étaient réduits d'environ le 145ième et le 450ième des concentrations observées dans le bassin directement arrosé. Le nombre total d'invertébrés benthiques sur des substrats artificiels dans le bassin directment arrosé a diminué tout au long de la période d'échantillonnage. Aucun déclin du nombre total des invertébrés n'a été observé dans le bassin protégé par la zone tampon. On a observé une mortalité variable chez les invertébrés en cage dans le bassin arrosé. ùes notonectes, les chironomidés et les tics ont été les plus durement atteints. Les invertébrés tenus encage dans le bassin

protégé par la zone tampon ont subi peu ou pas de mortalité. Trente pour cent des ombles de fontaine en cage dans le bassin arrosé sont morts avant l'interruption de l'étude alors qu'aucune mortalité n'a été observée chez les ombles du bassin protégé. Ces résultats indiquent que les zones tampons de 100 m sont nécessaires à la protection des voies d'eau contre les applications aériennes de deltaméthrine.

TOXICITY OF THE INSECT GROWTH REGULATOR DIFLUBENZURON TO STREAM INVERTEBRATES

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ABSTRACT

The recent outbreak of gypsy moth (*Lymantria dispar* L.) in Ontario and hemlock looper (*Lambdina fascillaria fascillaria* Guene) in Newfoundland have caused concern in the tourism and forestry industries. Natural control factors such as climate, disease organisms, parasites and predators have been responsible for the eventual decline of gypsy moth and hemlock looper populations, but additional control measures are required to reduce defoliation and thus tree mortality at the peak of infestations. Historically, aerial applications of insecticides have been used to protect foliage, but many of these chemicals are perceived by the public to be harmful to the environment, despite the fact they have been proven safe and effective. Thus there has been a trend towards the development of new insecticides with more environmentally and socially acceptable traits.

One group of insecticides are the insect growth regulators (IGR), which include the juvenile hormone mimics and the substituted benzoyl ureas (chitin synthesis inhibitors). One substituted urea, diflubenzuron, has been proven to be highly effective against lepidopterous pests, has low toxicity to vertebrates and has a relatively short half-life in the environment. However, due to its mode of action, concern has been expressed over the potential impacts of diflubenzuron on stream invertebrates.

This study determines the acute toxicity of diflubenzuron to a variety of stream invertebrates using a laboratory flow-through bioassay. The results are compared with those from similar laboratory studies using registered forestry insecticides.

RÉSUMÉ

La nouvelle recrudescence de la spongieuse (*Lymantria dispar* L.) en Ontario et de l'arpenteuse de la pruche (*Lambdina fiscellaria fiscelleria* Guene) à Terre-Neuve a causé de l'inquiétude dans l'industrie touristique et dans l'industrie forestière. Les mécanismes naturels de lutte contre ces défoliateurs comme le climat, les organismes pathogènes, les parasites et les prédateurs ont conduit au déclin des populations de sponieuses et d'arpenteuses, mais des mesures additionnelles de lutte sont requises pour atténuer la défoliation et ainsi la mortalité d'arbres au plus fort des infestations. Il y a longtemps que les insecticides sont pulvérisés du haut des airs pour

protéger le feuillage, mais beaucoup des agents chimiques utilisés sont perçus dans le grand public comme des substances nocives sur le plan écologique malgré que la preuvre de leur sécurité et de leur efficacité est faite. On a donc tenté de mettre au point de nouveaux insecticides ayant un profil écologique et social plus acceptable au grand public.

Les régulateurs de croissance des insectes constituent une catégorie d'insecticides; on y trouve des analogues d'hormones juvéniles et des benzoyl-urées à substitution (inhibiteurs de la synthèse de la chitine). Une urée à substitution, le diflubenzuron, s'est révélée très efficace contre les lépidoptères nuisibles, est peu toxique pour les vertébrés et a une demi-vie assez courte dans le milieu. Cependant, étant donné son mode d'action, des inquiétude ont été soulevées quant à ses effets potentiels sur les invertébrés des cours d'eau.

La présente étude en laboratoire a permis de déterminer une toxicité aiguë du diflubenzuron chez plusieurs invertébrés des cours d'eau grâce à des essais biologiques dans des enceintes à circulation permanente. Les résultats sont comparés à ceux d'autres études faites au laboratoire qui portaient sur des insecticides forestiers enregistrés.

SETTING REALISTIC CLEAN-UP STANDARDS FOR METALS: HOW CLEAN IS CLEAN?

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ABSTRACT

Recent United States legislation can require current and/or past owners of facilities to contain and/or clean up sources of pollutants contaminating ground and surface waters. This has been applied to areas of metals mining activity where sources can include mine drainage, tailings pile runoff or underflow, and smelter and refining wastes that have accumulated over many decades. In many cases, there have been attempts to enforce U.S. Environmental Protection Agency (EPA) criteria for the protection of aquatic life as the clean up standards to be met in adjacent surface waters. Frequently, such restrictive standards can only be achieved at extreme cost, if at all. Often, there are compelling factors indicating that cleanup to some standard less restrictive than the EPA criteria can allow recovery of fish populations to the maximum levels that could be supported in the absence of metal stresses resulting from the activities of man.

A typical problem is the absence of any data to describe metals levels prior to the advent of mining; in some cases, natural background levels may well have exceeded present-day criteria. Data are presented from an unmined area in Alaska where extremely high levels of metals occur naturally and where apparently healthy and reproducing populations of several salmonids are found in waters with mean dissolved zinc three to seven times the EPA chronic criterion. In another example from the

central Rocky Mountain region, populations of brown trout are reproducing in waters with dissolved zinc levels five or more times the criterion.

Several factors which may explain the disparity between these field results and laboratory derived criteria are explored:

(1) Acclimation of wild fish to chronic exposures and use of unexposed test fish in deriving criteria.

(2) Use of highly soluble salts in laboratory studies while a significant proportion of the even "dissolved" metals in field situations may be biologically unavailable.

(3) The fact that laboratory tests deal with effects on individuals while field data reflect populations with inherent compensatory mechanisms.

A methodology is described to use existing stream habitat models to predict salmonids densities supportable by the physical and hydraulic characteristics of the stream in the absence of metal stresses. Comparison of actual population sizes and associated metal levels is then used to estimate the level of metals that can be tolerated by the extant fish populations without apparent detriment. It is argued that these levels may constitute realistic cleanup goals for sites with major long-term contamination.

RÉSUMÉ

La législation récemment adoptée aux États-Unis peut obliger les propriétaires actuels et (ou) antérieurs d'installations à assurer la retenue et (ou) le nettoyage de sources de polluants qui contaminent le sol et les eaux de surface. Cette réglementation a été appliquée à certaines activités d'extraction de métaux dont les sources polluantes peuvent comprendre les eaux d'exhaure, le ruissellement ou l'écoulement souterrain que partent des tas de stériles et les résidues des hauts fourneaux ou de raffinage qui se sont accumulés pendant des dizaines d'années. Dans de nombre de cas, on a tenté d'utiliser les critères de la *Environmental Protection Agency* (*EPA*) des États-Unis pour la protection des formes de vie aquatiques comme normes devant être atteintes dans des eaux de surface contiguës aux sources de pollution. Fréquemment, des normes aussi sévères ne peuvent être atteintes qu'à un prix excessif, si elles peuvent jamais être atteintes. Souvent, on a de bonnes raisons de penser qu'un nettoyage jusqu'à un degré moins poussé que les critères de l'*EPA* peut permettre aux populations de poissons de retrouver les niveaux maximum qui peuvent être atteints lorsque les stress par des métaux d'origne anthropique sont supprimés.

Un des problèmes classiques est celui de l'inexistence de toute données sur les concentrations de métaux dans le milieu avant la mise en exploitation des mines. Dans certains cas, les concentrations de fond peuvent très bien avoir dépassé les seuils modernes. Nous présentons des données provenant d'une région d'Alaska qui n'a pas fait l'objet d'extraction minière et où on trouve des concentrations naturelles extrêmement élevées de métaux et où des populations de plusieurs salmonidés apparemment en bonne santé et qui se reproduisent sont trouvées dans des eaux contenant du zinc en solution dont la concentration est trois à sept fois celle qui sert de critère de toxicité chronique pour l'*EPA*. Dans un autre exemple de la partie centrale des Rocheuses, des populations de truite brune se reproduisent dans des eaux

contenant du zinc en solution dont la concentration est cinq fois supérieure ou plus à ce même critère.

Plusieurs facteurs susceptibles d'expliquer la disparité entre ces résultats sur le terrain et les critères obtenus en laboratoire, sont explorés:

1) L'accumulimatation de populations sauvages de poissons à une exposition chronique et l'utilisation de poissons non exposés pour les tests servant à l'elaboration des critères.

2) L'utilisation de sels très solubles dans les études de laboratoire tandis qu'une proportion importante des métaux même "dissous", sur le terrain n'est peut être pas absorbable par les organismes.

3) Le fait que les tests en laboratoire portent sur les effets observés sur des individus alors que les données sur le données sur le terrain portent sur des populations qui ont leurs mécanismes inhérents de compensation.

Une méthode est décrite pour l'utilisation de modèles existants d'habitat de cours d'eau pour prédire la densité de salmonidés supportable dans certaines conditions physiques et hydrauliques en l'absence de stress causé par des métaux. La comparaison des niveaux de population et des concentrations en métaux observées en même temps sert alors à l'évaluation de la concentration en métaux qui peut être tolérée sans dommage apparent par les populations existantes de poisson. Il est proposé que ces concentrations peuvent constituer des objectifs réalistes pour le nettoyage de certains secteurs fortement contaminés depuis longtemps.

ANALYSES DES COUTS-BENEFICES DE DIFFERENTS BIOESSAIS POUR UNE EVALUATION INTEGREE DE LA TOXICITE AQUATIQUE

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RÉSUMÉ

Pour choisir parmi la trentaine de bioessais courants en écotoxicité aquatique, le gestionnaire du contrôle de rejets en milieu aquatique a besoin d'une démarche articulée en fonction de quelques critéres tels que le coût impliqué, le délai de réponse, la portée de l'impact envisagé et al valeur de l'indication fournie par le test considéré. Il eput commencer par subdiviser sa démarche selon trois catégories d'écotoxicité envisagée, à savoir: létale, sous-létale directe et chronique, lesquelles correspondent à trois niveaux de protection environnementale et de solutions. Pour chacune de ces catégories, nous présentons les tests écotoxiques réguliérement utilisés au journ'hue, leurs durées, leurs volumes minimaux de rejet à échantillonner, leurs besoins en personnes techniques/jour et en analyses chemiques afin d'en précisser leurs coûts. Un tel exercice montre que les "microbioessais" à l 'échelle cellulaire (bactéries, algues unicellulaires et protozoaires) se révélent les plus avan-

tageux. Un seul type de bioessai ne peut cependant répondre à toutes les questions d'une évaluation écotoxicilogique d'un reject en milieu aquatique car cette évaluation doit associer causes et effets. Compte tenu de ces exigences, nous proposons une procédure progressive et sélective en trois étapes combinant analyses physicochimiques, bactériologiques et éecotoxiques. Sur une base de 10 series d'analyses, cette procédure a un coût moyen de $2,500 (minimum: $1,000 et maximum: $6,000) et apporte 4 fois plus d'informations de 8 types differents que celle effectuée au coût de $2,000 de facon relativement convenionnelle.

ABSTRACT

To select from about thirty bioassays currently being used in aquatic ecotoxicity studies, waste monitoring managers need a procedure based on criteria such as cost, response time, expected scope of impact and value of the test result. Managers could begin by dividing ecotoxicity into three catagories of lethal, directly sublethal, and chronic, that correspond to three levels of environmental protection and three kinds of solutions. For each category, we present the toxicity tests that are currently in regular use, with their durations, minimal sampling volumes, requirements for technicians per day, and requirements for chemical analyses, in order to determine their costs. The results show that "microbioassays" at the cellular level (bacteria, single-cell algae, and protozoans) are the best. However no single type of bioassay can answer all the questions that arise in evaluating the impact of a given dumping, because such an evaluation must relate cause and effect. We therefore propose a three-step selective procedure that combines physical-chemical, bacteriological, and ecotoxicological analyses. Assuming ten sets of analyses, the procedure has an average cost of $2500, with a minimum of $1000 and a maximum of $6000, yet it yields four times more information of eight types than the conventional procedure costing $2000.

MICRONUCLEI IN THE PERIPHERAL BLOOD OF *RANA PIPENS* AND THEIR USE IN AQUATIC TOXICOLOGY TESTS

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ABSTRACT

Chromosomal damage can be detected by an increased incidence of micronuclei in erythrocytes following division of damaged precursor cells. This micronucleus assay was originally developed in mammalian bone marrow, and then extended to the peripheral blood system which allows samples to be taken repeatedly from the same individual. This study extends the assay to a semi-aquatic species for relatively fast, inexpensive detection of mutagenic carcinogens in contaminated wetlands. The nuclei in *Rana pipiens*, unlike those of mammals, are not extruded from the erythrocytes, which may result in increased retention of micronuclei and better estimation of the extent of genetic damage.

Leopard Frogs, *R. pipiens*, were irradiated with 100, 200 and 400 rads per d (for five days on and two days off) for 21, 14 and 11 d, respectively. Peripheral blood samples were taken repeatedly from the toes of treated individuals and from untreated controls, smeared on slides, fixed in 100% methanol, and stained with Giemsa. With this technique, the nuclei and micronuclei stain purple, the normochromatic erythrocytes (NCE) stain pink and the polychromatic erythrocytes (PCE) stain blue. Induced micronuclei were first observed in PCEs on day 2 and increased somewhat over the next few days. The induced frequency was dose-related, but the dose response curve seemed to be non-linear. The NCE population did not respond within the study period.

RÉSUMÉ

L'endommugement des chromosomes peur être détecté par la présence accure de micronoyaux dans les érythrocytes formés apré la division de précurseurs anormaux. Á l'origine, ce test a été mis au point conformément aux principes de la formation de micronoyaux dans la moelle épinière de mammifères. Le test a ensuite été appliqué au système circulatorie périphérique, ce qui permettait la répétition des prises de sang chez un même sujet. Cette étude tentait d'appliquer le test à une espèce semi-aquatique et d'obtenir la détection assez rapide et peu coûteuse d'agents carcinogèneses mutagènes dans des terres humides contaminées. Á la différence de la situation chez les mammifères, les noyaux chez *Rana pipiens*, ne sont pas extraits des érythrocytes, ce qui peut se traduire par une rétention supérieure des micronoyaux et une meilleure évaluation de l'endommagement des chromosomes.

Des sujets de *R. pipiens* ont reçu 100, 200 et 400 rads par jours (pendant cinq jours suivis de deux jours sans traitement) pendant 21, 14 et 11 jours, respectivement. Des prises de sang à répétition omt été faites par les orteils des sujets traités et des témoins; le sang a été étalé sur lame. Après fixation au méthanol, les lames étaient colorées au giemsa. Avec cette technique, les noyaux et les micronoyaux sont colorés en pourpre, les érythrocytes normochromatiques (NCE) sont colorés en rose et

les érythrocytes polychromatiques (PCE) sont colorés en bleu. 224 partir des résultats obtenus, une évaluation de cette technique sera présentée.

INTRODUCTION

In recent years, there has been an interest in developing reliable assays capable of detecting environmental carcinogens. Various tests for genetic damage have been devised to measure induced mutations, chromosomal aberrations, or sister chromatid exchange. Widely used tests include the Ames assay, the sex-linked recessive lethal test, the dominant lethal test, and metaphase chromosome analysis (Hart and Armstrong 1984). Unfortunately, techniques such as these are either labour-intensive, or difficult to apply *in situ*.

In 1970, Heddle, and Schmid and his co-workers, independently developed a micronucleus assay in animals (Krepinsky and Heddle 1983). This assay takes less time than the metaphase analysis, is cheaper, does not depend on accurate timing, does not require highly trained technicians, and allows cumulative cytogenetic damage to be assessed since micronucleated erythrocytes persist in the circulation (Heddle et al. 1983).

The micronucleus (or Howell-Jolly body) is a small cytoplasmic body that is produced when chromosomes or acentric chromosomal fragments lag at anaphase (Schlegel and MacGregor 1982). The micronuclei resemble the nucleus, with the same structure, shape and intensity of staining, but they are smaller in size. They can be clearly seen in the cytoplasm of erythrocytes close to the main nucleus. Their increased frequency after exposure to a clastogenic agent is dependent on the mitotic rate, the variation in frequency of acentric chromosomal fragments, the number of acentric fragments that give rise to micronuclei, the life span of the cells, and the selective pressure against cells with micronuclei (Salamone and Heddle 1983).

In the peripheral blood, the micronuclei are first apparent in the polychromatic erythrocytes (PCEs) or reticulocytes of red blood cells (Barale et al. 1985). The PCEs mature into normochromatic erythrocytes (NCEs). The PCEs can be identified by the staining property of residual ribosomal RNA. With Giemsa stain, the frog PCE cytoplasm stains bluish-grey and appears to have a slightly rougher texture than the NCE cytoplasm. In contrast, the NCE cytoplasm stains pinkish-grey. Both the PCEs and the NCEs contain an oval nucleus which stains a dark purple with Giemsa.

Micronucleus assays have been developed using both the bone marrow, as well as the peripheral blood. A variety of organisms have been used, including axolotls (Jaylet et al. 1986a) newts (Jaylets et al. 1986b), mice (Choy et al. 1985), fish (Hooftman and de Raat 1982) and rats (Schlegal and MacGregor 1984). It was the purpose of this study to examine frogs as a possible test system for detecting genotoxic pollutants in freshwater.

Frogs have several potential advantages as micronucleus test organisms: (1) Their erythrocytes are nucleated, so, presumably, the micronucleated cells are not selectively removed by the spleen as they are in rats and humans (Schlegal and MacGregor 1984; Schlegal et al. 1986). The micronucleus is retained in the cell, which then enters the peripheral blood system where it can be easily sampled, as in mice. (2) The blood volume of the organism is sufficient in relation to the negligible volume required for analysis, so the organism need not be sacrificed. Therefore,

multiple samples can be taken from the same animal, and each animal can serve as its own control. (3) The species used (*Rana pipiens*) is ubiquitous in North America, and can, therefore, be collected from many different areas for comparison of genetic toxicity *in situ*. (4) Amphibians are fairly sensitive to x-rays due to their relatively high nuclear volume and interphase chromosome volume (Sparrow 1970; Conger and Clinton 1973). This implies that the frogs may respond quickly to low doses of x-radiation.

MATERIALS AND METHODS

Adult Leopard Frogs (*R. pipiens*) were collected from the Caledon East area, north of Toronto, Ontario. The frogs were kept in plastic bread crispers, which were perforated top and bottom and partly submerged in plastic mouse cages filled with tap water. The water was changed at last once a wk and no more than four frogs were held in each container. Only apparently healthy individuals were used. The frogs were allowed two wk to acclimatize to laboratory conditions, at room temperature in a lab with natural lighting. They were fed once a wk on live lab-reared crickets and locusts.

The frogs were irradiated in a Phillips constant potential industrial x-ray system using 160 kv and 18.6 mA. They were divided into four groups, each consisting of a small, medium and large frog. The group mean weights at the time of initial treatment were 19.0-23.2 g. The three treatment groups received 100 rad, 200 rad and 400 rad on an almost daily basis, and the fourth group served as a control. Each frog could be identified by colour pattern and weight. The organisms were weighed each time they were sampled. They were irradiated 19 times over a period of 25 d, and sampled 11 times. The frogs were restrained during irradiation in a perforated plastic bottle and returned to their cage as quickly as possible to prevent dehydration.

Blood smears were made by clipping one of the toes of the frog. This allowed for repeated samples from the same specimen. The blood was then smeared on a slide using another slide to spread the cells as evenly as possible. The slides were allowed to air-dry, and were then fixed in 100% methanol for 20 min. They were allowed to dry prior to staining in 5% Giemsa stain in Sorensen's buffer and rinsed with distilled water. The slides were left to dry for 24 h and then mounted using DPC mountant. In order to avoid coagulation during preparation, a small drop of foetal bovine serum was used to enhance spreading of the smear and prevent quick drying and clumping.

The slides were randomized and coded prior to coding. The incidence of micronucleated cells among PCEs and NCEs was determined at 1,000 times magnification under oil immersion by two individuals. One thousand normochromatic erythrocytes were scored from each sampling. The ratio of polychromatic to normochromatic cells was determined to monitor possible cytotoxic effects of chronic treatment on erythropoietic tissues (Heddle et al. 1984). The total number of PCEs with micronuclei was normalized by the number of PCEs scored, to obtain the frequency of micronucleated cells per 1000.

The following criteria were used for scoring: (1) the micronucleus had to have the same texture and colour as the nucleus of the cell in which it was located; (2) it

could not refract light at the level of the nucleus; and (3) objects like it could not occur in larger numbers outside the cell than inside.

Adhering rigidly to these guidelines prevented dirt and bacteria from being counted as micronuclei. Dirt particles were a different colour than the nucleus, were irregularly shaped, and refracted light so that they appeared white when the cell was brought in and out of focus. In the Leopard Frog, outcroppings of the nucleus were observed, ranging from finger-like projections to round structures attached by a thin thread to the nucleus. These structures were not counted as micronuclei.

RESULTS AND DISCUSSION

A time-dependent increase in the frequency of micronucleated PCEs was observed following whole-body irradiation at sublethal doses. Fig. 1 shows that, except for the 200 rad group, relatively large increases of micronuclei were obtained in the PCEs after two d, and all treatment groups were increased relative to the control after five d of daily irradiation. The corresponding data are listed in Table 1. The groups that received 100 rad and 200 rad daily had approximately the same number of micronucleated cells per 1000 PCEs. Both groups experienced an unexplained drop in the percentage of micronucleated cells on Day 11. This was not observed, however, in the 400 rad group, which showed the greatest radiation response, with a plateau by day 11. The percentage of PCEs in the peripheral blood pool does not suggest erythropoietic depression (Table 2).

Table 1. Micronucleus (MN) frequency/1000 PCEs scored after daily irradiation of frogs with different doses of x-rays. (1000·total MN per treatment group/total PCEs per group)

Treatment	Day 0	Day 2	Day 5	Day 9	Day 11
Control	4.07	2.9	4.1	0	3.4
100 Rad	0	18.4	21.3	27.0	14.6
200 Rad	1.6	2.8	26.4	25.1	9.4
400 Rad	0	21.3	32.0	37.9	37.7

Table 2. Percentage of PCEs in peripheral erythrocyte pool. (100.total PCEs per treatment group/total NCEs plus PCEs per group).

Treatment	Day 0	Day 5	Day 9	Day 11
Control	7.5	11.0	8.5	8.5
100 Rad	6.1	10.7	9.7	10.1
200 Rad	7.6	8.2	4.0	5.4
400 Rad	1.8	6.0	2.5	1.7

There was no change in frequency of micronucleated NCEs over the study period (Fig. 2, Table 3), and all three treatment groups remained below a background level of three micronucleated NCEs per 1000. The NCEs are older cells, which have

developed from the PCEs, and are part of a much larger cell pool. It may take a considerable period of time for damage induced in the PCEs to accumulate to a significant level in the NCE pool.

Table 3. Micronucleus (MN) frequency/1000 PCEs scored after daily irradiation of frogs with different doses of x-rays. (1000·total MN per treatment group/total PCEs per group)

Treatment	Day 0	Day 2	Day 5	Day 9	Day 11
Control	0	0	2.7	1.1	0
100 Rad	0	0	0.7	2.8	0
200 Rad	0.8	0	0	0.4	0.6
400 Rad	1.8	0	1.5	1.2	1.4

Table 4. Comparative sensitivity of different organisms to x-radiation based on micronucleus response.

Reference	Treatment (days)	Organism	Number of micronucleated RBCs/1000	NCEs/1000	PCEs/100
Jenssen and Ramel (1978)	200 R (1)	Mouse	-	1.7	-
Jenssen et al. (1984)	100 R (1)	Mouse	-	-	45.5
Siboulet et al. (1984)	120 R (1)	Newt	120	-	-
Present study	100 R (5)	Frog	-	0.7	21
	200 R (5)	Frog	-	0.0	27
	400 R (5)	Frog	-	1.5	32
Dinnen et al.(This vol.)	50 R (5)	Fish	-	1.9	14.2[1]
	100 R (5)	Fish	-	0.0	23.0[1]
	400 R (5)	Fish	-	1.4	61.2[1]

Fig.3 shows the PCE dose response curve five d after initiation of treatment. In controls, the frequency of micronucleated cells was 0.41%. At a concentration of 100 rad, the frequency was significantly elevated to 3.12%. The dose response curve indicates that, even at 25 rad for five d, we should be able to see a doubling of the frequency of micronucleated cells relative to controls.

The sensitivity of the frog test system can be compared with that of other test organisms. Some comparisons are shown in Table 4. It appears that the frog test system is less sensitive than the mouse (Jenssen et al. 1974; Jenssen and Ramel 1978), although exposure regimens and cell populations tested differ among studies. It is also less sensitive than the fish test based on PCE-precursor cells (Dinnen et al., this volume). However, the test system may be of use in certain semi-aquatic habitats where fish cannot be used.

A number of questions remain to be resolved. In particular, the lack of NCE response over the study period leaves the duration of the PCE cell stages in question. This would be important in interpretation of results from long-term *in situ* exposures, since it determines the exposure period corresponding to the measured response.

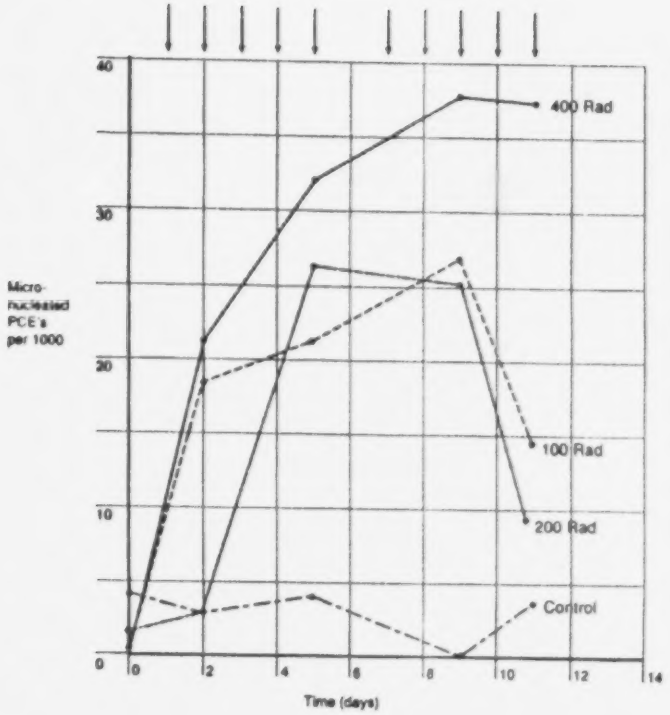

FIGURE 1
Increase in Micronucleus Frequency in PCE with Time for Three Chronic Radiation Doses in Rana pipiens

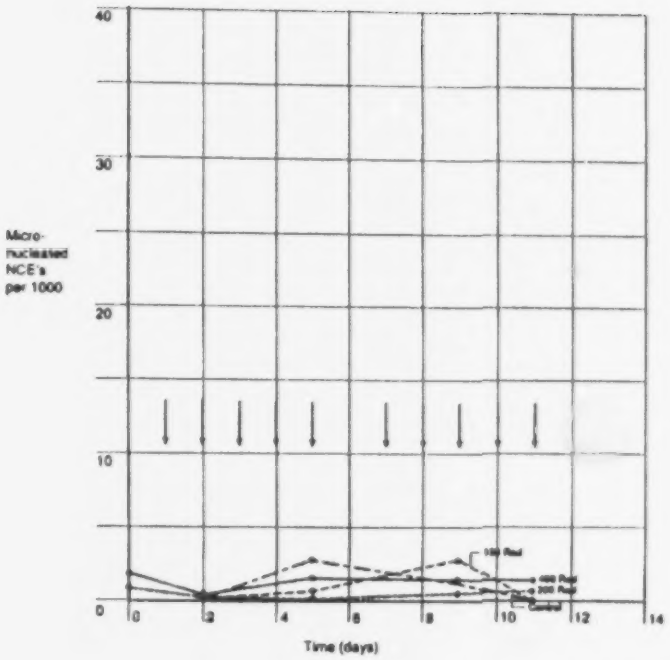

FIGURE 2
Increase in Micronucleus Frequency in NCE with Time for Three Chronic Radiation Doses in Rana pipiens

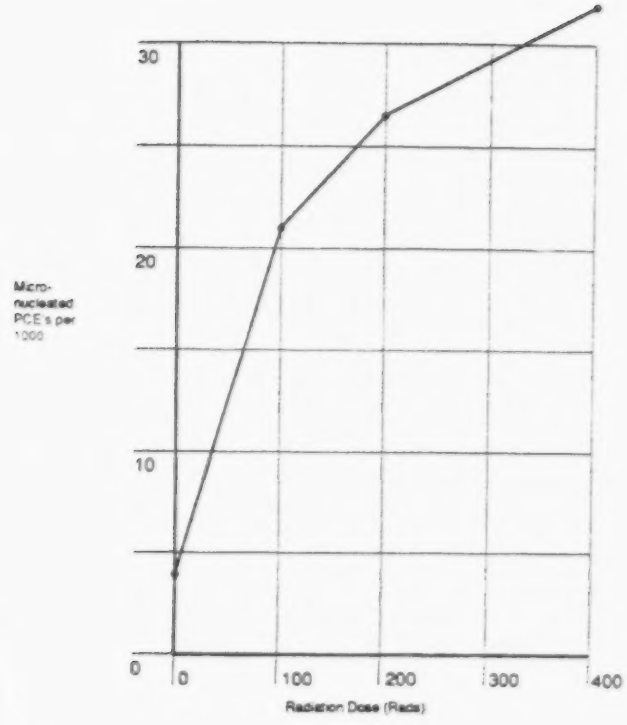

FIGURE 3
Dose Response Curve for Chronic X-Radiation of Rana pipiens Five Days After First Treatment

ACKNOWLEDGEMENTS

This work was supported by the Natural Sciences and Engineering Research Council of Canada.

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APPLICATION OF A MICRONUCLEUS ASSAY TO THE PERIPHERAL BLOOD CELLS OF THE RAINBOW TROUT, *SALMO GAIRDNERI*

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ABSTRACT

The extension of the micronucleus assay to the peripheral blood of the rainbow trout, *Salmo gairdneri*, may permit easy detection of clastogenic (chromosome breaking) agents present in aquatic environments. Radiation, a known clastogen, was used to investigate the sensitivity and timing of formation of micronuclei in the blood, and hence to indicate the utility of the test for the measurement of chromosomal damage.

The experimental design involved the irradiation of fish every 24 h at specified doses ranging from 50 to 400 rads. Blood samples, taken periodically from the different treatment groups, were smeared on slides and stained in 5% Giemsa stain. This allowed differentiation between the polychromatic erythrocytes (PCEs) and the normochromatic erythrocytes (NCEs). The slides were subsequently scored for the presence of micronuclei in both PCEs, in a class of cells that we term X-cells (probably PCE precursors), and NCEs.

Radiation treatment caused a significant increase in micronucleated erythrocytes in the X-cell class after 48 h, in the PCE class after 5-7 d, and in the NCE class after 17-23 d.

The X-cell response was enhanced over the response that was seen in the PCEs or NCEs, indicating that the X-cells could be used to detect a much lower radiation dose. The results of this study indicate that the rainbow trout system could provide a very sensitive and rapidly performed *in situ* test for the presence of water-borne clastogenic agents.

RÉSUMÉ

L'application du test des micronoyaux au sang prélévé dans la partie périphérique du système circulatorie de la truite arc-en-ciel, *Salmo gairdneri*, facilitera peut-être la détection d'agents clasogènes présents dans les milieux aquatiques. Les radiations, un agent efficace de bris de chromosomes, servira à l'étude de la formation des micronoyaux dans le sang; cela pumettra de noir quelle est l'utilité du test comme moyen d'identifier les dommages subis par les chromosomes.

Le protocole d'expérience comprend l'irradiation de poissons tous les 24 h à des doses précises comprises entre 50 et 400 rads. Des prises de sang seront faites périodiquement dans les différents groupes de traitement et le sang sera étalé sur lame et coloré au giemsa ouà l'acridine orange pour des tests au microscope à fluorescense. Chaque colorant permet de distinguer les érythrocytes polychromatiques (PCE) des érythrocytes normochromatiques (NCE). Une cote Sera attribueé aux lames selon qu'on y observe des micronoyaux dans les PCE comme dans les NCE, afin d'en déterminer la frèquence.

Des courbes dose-effet seront présentées selon chaque période de traitemenet er des comparaisons seront établies afin de déterminer le moment de l'effet maximal. La valeur de ce système comme moyen d'évaluer la contamination du milieu se trouverait montrée par une fréquence accure des micronoyaux dans les PCE des groupes traités par rapport aux résultats observés dans les groupes témoins. Le test sera évalué à partir des résultats obtenus.

INTRODUCTION

Micronuclei are bodies which have similar shape, structure, and staining properties to the main nucleus, but range from 1/20 to 1/5 its size (Krepinsky and Heddle, 1983). They may be formed in certain cells after chromosomal damage by treatment with a clastogenic agent. Assays have been developed which can measure this damage, as shown by an increase in the frequency of cells possessing micronuclei. Such tests include the mouse bone marrow assay (Heddle 1972; Heddle et al. 1984) or the human peripheral blood lymphocyte assay (Krepinsky and Heddle 1983; Countryman and Heddle 1976). These tests, however, are designed for testing isolated compounds. They cannot conveniently be used to measure the genotoxicity of chemical agents in water at the prevailing ambient concentrations. A test is required which utilizes an organism indigenous to the aquatic environment. We therefore present an *in vivo* test utilizing the peripheral blood of the rainbow trout, *Salmo gairdneri*.

The trout peripheral blood assay has the potential advantages of existing micronucleus assays, such as speed and economy, and the further advantage that only peripheral blood samples are required (MacGregor et al. 1980). The rainbow trout has been used extensively in previous toxicological studies (Dalich et al. 1981; Van Der Putte et al. 1981; Cleveland and Hamilton 1983; Al-Sabti 1985). Hence, it is easily available and biologically well characterized.

In previous studies of micronucleus induction in fish peripheral blood erythrocytes (Hooftman and de Raat 1982; Das and Nada 1986), no distinction has been made between newly formed polychromatic erythrocytes and older normochromatic erythrocytes. This distinction would permit recent effects to be distinguished from those of long-term exposure (Heddle et al. 1984).

In the present study, x-radiation, which is a known clastogen (Krepinsky and Heddle 1983), was used to induce micronuclei in fish peripheral blood cells. This gave an indication of the timing and sensitivity of the system in order to determine its practicality for use in the field. Polychromatic erythrocytes and normochromatic erythrocytes were separately scored for micronuclei. In addition, a distinct class of cells (termed X), that appeared to be PCE precursors, were observed and scored. They were found to be the most sensitive to radiation treatment, and to respond more quickly than either the polychromatic or normochromatic erythrocytes.

MATERIALS AND METHODS

Seventy rainbow trout acquired for Aquafarms Inc., and ranging in weight from 2-10 g (determined at sampling time) were divided into three treatment groups, and one control group. The fish were exposed to 50, 100, and 400 rads on 16 of 23 days as shown in Fig. 1. The control fish were handled similarly to the treated fish, but without exposure to radiation. Two fish were sacrificed from each treatment and control group at periodic intervals over the exposure period.

Each fish was irradiated by placing it in a screw-top plastic centrifuge tube. The 50 rad group was exposed for 0.3 min, the 100 rad group for 0.6 min, and the 400 rad group for 2.4 min at 18.6 mA and 160 kv, approximately 38 cm directly below the x-radiation source. The machine was a Phillips constant potential industrial x-ray system.

The trout to be sampled were first weighed on a Mettler PC 4400 top-loading balance. They were then anesthetized in a 1 g/100 mL solution of MS-222 (3 Amino-benzoic Acid, 4 Ethyl Ester) Sigma #A-5040 in water. After 5-10 s, the tail was severed, a drop of blood placed on a slide and evenly spread. After sampling, the slides were allowed to air-dry, fixed in 100% methanol for 15 min, and then stained in 5% Giemsa in 1M/15 Sorensen's buffer for 15-20 min. Following staining, the slides were rinsed once with buffer and once with double distilled water, air dried and mounted with DPX mountant.

After randomizing and coding the slides, normochromatic erythrocytes (NCEs), polychromatic erythrocytes (PCEs), and X-cells were scored for micronuclei under oil immersion at 1000 X magnification. The number of micronucleated PCEs in a total of 500 PCEs was recorded. The number of NCEs observed among the first 100 PCEs was also recorded, along with the number of micronucleated NCEs. The number of X-cells found among the 500 PCEs was also recorded, along with the number of micronucleated X-cells. From these data, the NCE:PCE ratio, and the proportions of micronucleated cells among the X-cells, PCEs and NCEs were calculated at each sampling time.

RESULTS

Fig. 1 indicates the frequency of micronucleated cells observed in the polychromatic erythrocytes on specified days following commencement of treatment. The arrows indicate the days on which irradiations were performed. Each point corresponds to the scoring of 500 PCEs from an individual fish. A clear increase can be noted in the frequency of micronucleated erythrocytes in those fish given 100 and 400 rads daily. On day 23, however, very few PCEs were found in samples from some individuals. Only 13 PCEs were found for every 1000 NCEs in one fish from the 100 rad treatment group. In addition, 15 and 14 PCEs were found for every 1000 NCEs in two fish from the 400 rad treatment group.

In the 50 rad treatment group, no clear increase in the frequency of micronucleated erythrocytes is evident. However, a cumulative frequency curve, based on the number of fish in which a specified number of micronucleated cells were noted, suggests that this group is different from the control (Fig. 2).

A distinct class of cells were recognized which were unlike either PCEs or NCEs. These cells (termed X-cells), stained darker blue, were rounder in shape, and the nucleus occupied a greater proportion of the cytoplasm than in PCEs. Since micronuclei were frequently observed in these cells, they were scored separately to determine their significance. Fig. 3 illustrates the frequency of micronucleated cells found for every 500 X-cell scored, and shows that there is a more rapid and enhanced response in X-cells compared to the PCEs in all three treatment groups. The fluctuation in frequency of micronucleated cells seen in some X-cell curves results from the low proportion of X-cells compared to PCEs.

Fig. 4 illustrates the NCE response based on scoring of 11 NCEs found in the first 100 PCEs scored. In all groups, no response was noted until day 23. The two day-23 points in the 400 rad group are based on 1000 NCEs each, since very few PCEs were observed.

Tables 1-3 show the exact counts of micronucleated cells in control and treatment groups on each sampling day, for PCEs, X-cells, and NCEs, respectively. The counts of X-cells and NCEs are normalized to a sample of 500 cells scored, since the actual number of cells scored was variable.

Table 1. Micronucleated cell frequency in 500 polychromatic erythrocytes with time and radiation dose.

Day	Control	50 Rad	100 Rad	400 Rad
0	0	-	-	-
1	1	0	0	1
1	1	0	0	1
2	2	0	0	0
2	5	0	0	0
3	3	0	2	0
3	1	1	2	1
5	4	3	0	4
5	1	0	0	2
7	0	7	0	44
7	0	2	4	7
9	0	5	4	11
9	0	4	5	45
13	0	0	12	28
13	3			10
17	1	2	14	23
17	1		6	37
23	1	4	0/13*	0/15*
23	1	4	17	0/14*

*500 PCEs could not be found for scoring.

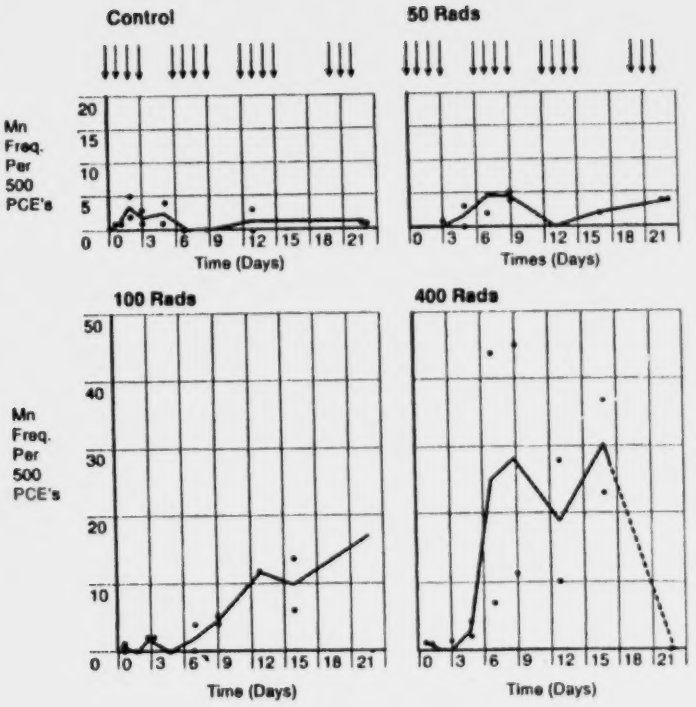

FIGURE 1
Change in Micronucleus Frequency in Polychromatic Erythrocytes with Time and Chronic Radiation Dose

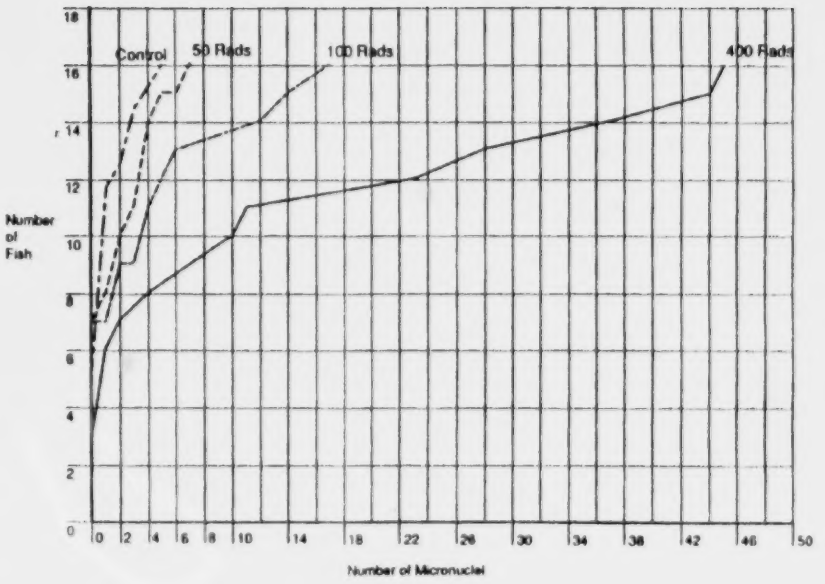

FIGURE 2
Cumulative Frequency Curves of Micronuclei for Polychromatic Erythrocytes in Control and Three Chronic Radiation Treatments

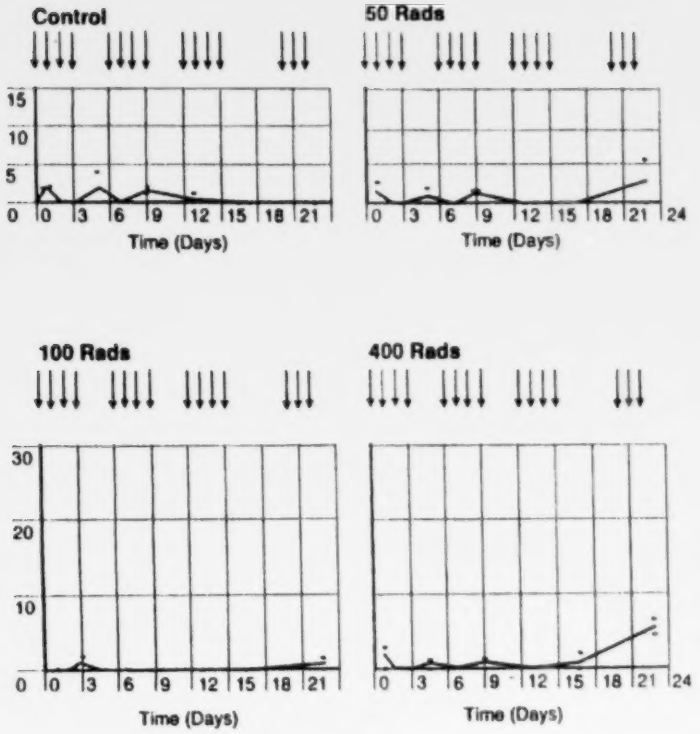

FIGURE 3
Change in Micronucleus Frequency in Normochromatic Erythrocytes with Time and Chronic Radiation Dose

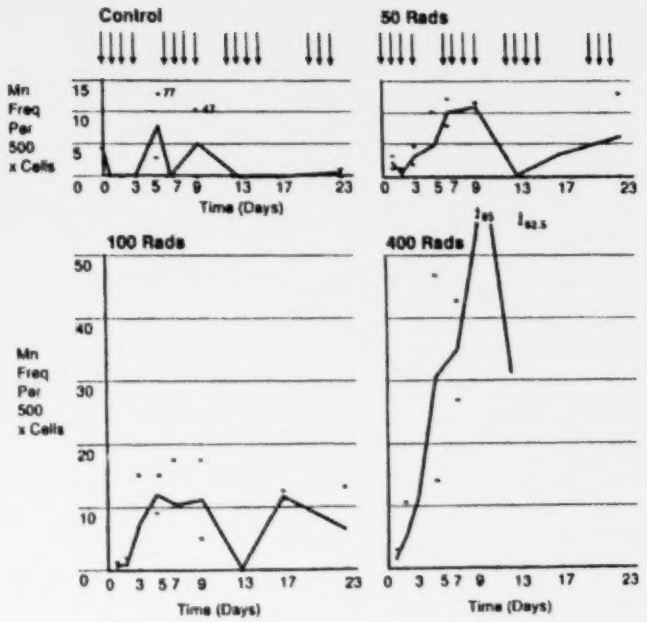

FIGURE 4
Change in Micronucleus Frequency in X-Cell Erythrocytes with Time and Radiation Dose

DISCUSSION

The increase in the frequency of micronucleated cells among polychromatic erythrocytes of the rainbow trout observed after exposures to x-rays (Fig. 1) was expected from the behaviour of the mouse bone marrow system (MacGregor et al. 1980). Since PCEs are newly formed erythrocytes, the induced micronuclei should be observed early in these cells.

Micronuclei appeared in the X-cell population sooner after irradiation than in the PCE population, suggesting that the X-cells may be precursors to the PCEs. On days 13-23 in the 400 rad treatment group, only 0-16 X-cells were noted for every 500 PCEs scored. If the X-cells are PCE precursors, it is likely that radiation had virtually arrested erythropoiesis causing a decline in the number of X-cells first.

In the 100 rad treatment group, the frequency of micronucleated cells in the X-cell population increased very quickly 48 h after the commencement of treatment, and reached a plateau at 72 h. In the PCEs, an increase was first observed 7-9 d after the start of treatment, and reached a plateau after 13-15 d. It is likely that the micronucleated X-cells had become micronucleated PCEs by this time. X-cells then could serve as faster and more sensitive indicators of clastogenic-mutagenic agents than either PCEs or NCEs.

Table 2. Micronucleated cell frequency in X-cell erythrocytes with time and radiation dose.

	Control			50 Rad			100 Rad			400 Rad		
Day	Cells scored	mn	mn 500	Cells scored	mn	mn 500	Cells scored	mn	mn 500	Cells scored	mn	mn 500
0	338	3	4.4	-	-	-	-	-	-	-	-	-
1	100	0	0	158	1	3.2	211	0	0	140	0	0
1	494	0	0	351	1	1.4	351	1	1.4	166	1	3
2	84	0	0	402	1	1.2	229	1	2.2	201	0	0
2	178	0	0	313	0	0	158	0	0	191	4	10.5
3	234	0	0	269	1	1.9	115	0	0	176	4	11.4
3	155	0	0	534	5	4.7	168	5	14.9	208	25	60.1
5	170	1	2.9	142	0	0	277	5	9.0	245	7	14.3
5	77	2	13.0	350	7	10.0	200	6	15.0	245	23	46.9
7	96	0	0	250	4	8	151	1	3.3	132	7	26.5
7	102	0	0	81	2	12.3	170	6	17.6	70	6	42.9
9	362	0	0	300	7	11.7	142	5	17.6	136	23	84.6
9	47	1	10.6	198	4	10.1	102	1	4.9	35	6	85.7
13	184	0	0	257	0	0	107	0	0	16	2	62.5
13	390	0	0							0	0	0
17	172	0	0	139	1	3.6	165	4	12.1	2	0	0
17	105	0	0				139	3	10.8	3	0	0
23	205	0	0	101	0	0	0	0	0	10	0	0
23	431	1	1.2	307	8	13.0	194	5	12.9	0	0	0

In the NCEs, an increase in the frequency of micronucleated cells was evident on day 23 only. Samples up to day 17 showed no increase in the frequency of micro-

nucleus formation. This lag was again expected from the behaviour of other systems (Heddle et al. 1984), even though the timing of erythropoiesis in rainbow trout was unknown. Since NCEs are older cells, a lag in the micronucleus response should exist because the cells that are affected at mitosis require a certain length of time to reach the NCE stage. From these data, the rainbow trout erythrocytes become NCEs after 17-23 d.

The sensitivity of this assay is indicated by the marginal results at 50 rads. One factor influencing this sensitivity is the animal-to-animal variation. The fish used were randomly bred animals, not inbred stains, and so genuine differences between animals may be expected. To determine whether the variation was caused by the scoring, the slides from day 7 in the 400 rad treatment group were re-scored at a different location on the slide. The new scores were similar to scores previously obtained, indicating that the observed differences between fish were unlikely to be a result of a chance distribution of micronucleated erythrocytes on the slides.

Table 3. Micronucleated cell frequency in normochromatic erythrocytes with time and radiation dose.

	Control			50 Rad			100 Rad			400 Rad		
Day	Cells scored	mn	mn 500	Cells scored	mn	mn 500	Cells scored	mn	mn 500	Cells scored	mn	mn 500
0	387	0	0	-	-	-	-	-	-	-	-	-
1	238	1	2.1	281	0	0	295	0	0	164	1	3
1	512	2	2.0	195	1	2.6	179	0	0	278	0	0
2	192	0	0	331	0	0	152	0	0	292	0	0
2	396	0	0	288	0	0	193	0	0	206	0	0
3	319	0	0	368	0	0	199	0	0	258	0	0
3	126	0	0	607	0	0	349	1	1.4	307	0	0
5	248	2	4.0	304	0	0	198	0	0	324	0	0
5	202	0	0	225	1	2.2	221	0	0	375	1	1.3
7	235	0	0	272	0	0	341	0	0	261	0	0
7	171	0	0	255	0	0	200	0	0	257	0	0
9	386	1	1.3	344	1	1.5	241	0	0	400	1	1.2
9	283	1	1.8	359	1	1.4	184	0	0	145	0	0
13	471	1	1.1	279	0	0	355	0	0	415	0	0
13	198	0	0							343	0	0
17	315	0	0	183	0	0	228	0	0	263	1	1.0
17	310	0	0				154	0	0	423	0	0
23	167	0	0	178	0	0	1000	3	1.5	1000	13	6.5
23	166	0	0	368	4	5.4	420	0	0	1000	9	4.5

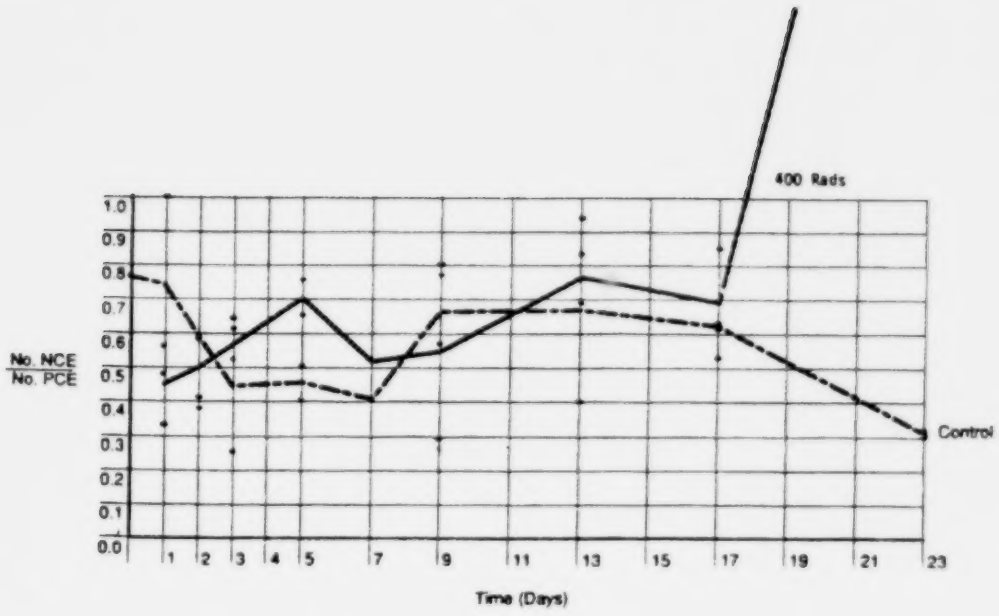

FIGURE 5
Measurement of Cyto-toxicity using NCE/PCE Ratio

The trout system has the advantage of providing a very sensitive and rapidly performed test for measuring short-term clastogenic effects using X-cells, as well as a test for longer term effects using PCEs or NCEs.

A more accurate quantification of timing of the process, as well at its sensitivity to actual water-borne agents is required before it can be of practical use in the field. When such questions have been adequately answered, the rainbow trout may prove to be an excellent system for detecting genotoxic pollutants.

ACKNOWLEDGEMENTS

We thank C. Urlando for his helpful assistance in the laboratory. This project was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

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CHROMOTEST - A COMPARATIVE REVIEW

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ABSTRACT

The environmental science community is very interested in quick and inexpensive short term bacterial mutagenicity tests because they see the possibility of monitoring air and water samples for potential mutagenic and carcinogenic agents. While the Ames Assay is the bacterial assay most often used by genetic toxicologists, several Canadian environmental laboratories have been using another bacterial mutagenicity test called the SOS Chromotest.

The SOS Chromotest is a rapid assay, taking one day to run. So far, results have been published with 245 chemicals. At least 18 laboratories worldwide have used the test. The overlap with the Ames/*Salmonella* test for 180 chemicals is 90%. While the total number of chemicals tested is not high, there are several classes of chemicals for which the SOS Chromotest has been shown to be almost 100% overlap with the published Ames Assay data. In a comparison with animal carcinogenicity data from a small subset of chemicals (56) in the EPA Gene-Tox Carcinogen Data Base, SOS Chromotest and the Ames Assay gave very similar predictions of animal carcinogenicity. As more chemicals are tested, if the high correlation between the Ames Assay and the Chromotest continues, it appears that the justification for using one or the other of these two tests will depend on the value of the information gained in relation to the cost of obtaining that information.

RÉSUMÉ

La communauté scientifique de l'environnement s'interesse aux essais de mutagénicité bacterienne à court-terme qui sont rapides et peu dispendieux. Ces essais peuvent être utilisés pour la surveillance des échantillons d'air et d'eau pour des agents potentiellement mutagéniques et carcinogéniques. Même si l'essai d'Ames est le plus utilisé par les toxicologists génétiques plusieurs laboratoires Canadiens d'environnement utilise un autre essai de mutagénicité bacterienne qui s'appelle 'SOS Chromotest'.

Le 'SOS Chromotest' est un eassai rapide qui ne prend qu'une journée a executé. A ce jour, il y a eu des résultats publiés sur 245 produits chimiques. Un minimum de 18 laboratoires das le monde utilise cet essai. Pour 180 produits chimiques il y a une coincidence de 90% entre l'essai d'Ames/*Salmonella* et le 'SOS Chromotest'. Il faut noté que les produits chimiques évalués avec le 'SOS Chromotest' ne sont pas nombreux, quand même il y a plusieurs classes de produits chimiques pour lesquelles l'essai 'SOS Chromotest' a une coincidence de ores de 100% avec les résultats publiés de l'essai a'Ames. Dans une comparaison avec la carcinogénicité animale, les données abtenues pour 56 produits chimiques, vennant d'une petite partie de 'EPA Gene-Tox Carcinogen Data Base', ont demontré que les résultats obtenus de l'essai 'SOS Chromotest' et celui d'Ames sont trés semblables pour des predictions de carcinogénicité animale. Si la correlation entre les essais Ames et 'SOS Chromotest' continue, au fur et à mesure que d'autre produits chimiques sont évalués, il apparait que le choix de l'un ou l'autre de ces essails dépendra de la valeur des renseigne-

ments obtenus versus le coût pour obtenir ces renseignements.

SCREENING SEDIMENTS FOR TOXICITY: A WATER-CONCENTRATION RELATED PROBLEM

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ABSTRACT

Forty-two sediments have been sampled in the Dutch sedimentation areas of the river Rhine. The sediments were classified on the score of measured contaminant concentrations, after a correction for sediment characteristics. Several bioassays procedures with sediments and extracted pore waters were carried out to assess the potential toxic effects associated with the contaminants present. The results indicated the importance of macro-elements, e.g. ammonia and salts, which should be measured as additional parameters to distinguish them from other toxicants as potential causative agents. In general the most sensitive information was obtained in the bioassays using the sampled pore waters. Therefore standard test organisms in aquatic toxicology seem to be well applicable to screen sediments for their potential toxic qualities. Additional experiments with *Daphnia magna* and *Chironomus riparius* in sediments, artificially contaminated with Cd, Hg and Dieldrin, supported the hypothesis that the main route of contaminant uptake by aquatic organisms is the one via the (pore)water, also when sediment inhabiting species are involved. Nevertheless the relevance of generating data on (epi)benthic organisms is argued.

RÉSUMÉ

42 échantillons de sédiments aquatiques ont été pris dans aires de sédimentation du delta du Rhin. Les sédiments ont été classifiés selon les concentrations de pollutants mesurés, après avoir fait une correction tenant compte des caractéristiques du sédiment en question. Plusieurs tests biologiques ont été appliques aux sédiments et à l'eau de pore extraite du sédiment pour déterminer les effets toxiques dûs aux divers contaminants. Ces résultats ont démontré l'importance de certains éléments, comme l'ammoniaque et divers sels, qui doivent être analysés pour établir leur contribution éventuelle à effet toxique. En général, les tests effectués dans l'eau de pore apparaisent être les plus sensibles. De ce fait, les tests standardisés de toxicologie aquatique semblent être fort bien utilisables pour mesurer rapidement le potentiel toxique d'un sédiment. Des tests additionels avec *Daphnia magna* et *Chironomus riparius* dans des sédiments contaminés artificiellement avec Cd, Hg ou du Dieldrin confirment l'hypothèse que la route principle pour absorber des contaminants est celle par l'eau de pore, même dans cas des espèces résidantes dan le sédiment. Néanmoins, la nécessité d'agrandir notre connaissance des espèces (epi)benthiques a été argumentée.

INTRODUCTION

The majority of the experimental work in aquatic toxicology has been focussed on the potential effects of contaminants upon pelagic organisms. For regulatory purposes, data on the acute toxicity to algae, crustaceans and fishes form a basic framework (OECD 1981).

Because of the growing awareness of sediment contamination, especially in industrial and sedimentation areas, the need has arised to gather information on the potential effects of polluted sediments upon (epi)benthic organisms. Fig. 1 illustrates how the different compartments of a sediment-water system might contribute to the contaminant uptake by these organisms. Based upon the results of field studies (van Urk and Kerkum 1987), midge larvae (Chironomidae) among others, have been selected as test organisms. Standardized assays are being developed to identify and quantify pollutant-specific effects and to screen sediment samples for their potential environmental impact (e.g. LeBlanc et al. 1985; Ziegenfus et al. 1986). On a relative short term, ecotoxicologically relevant information can be generated. A conceptual model (Fig. 2) shows how these bioassays fit in an overall impact assessment of contaminated sediments.

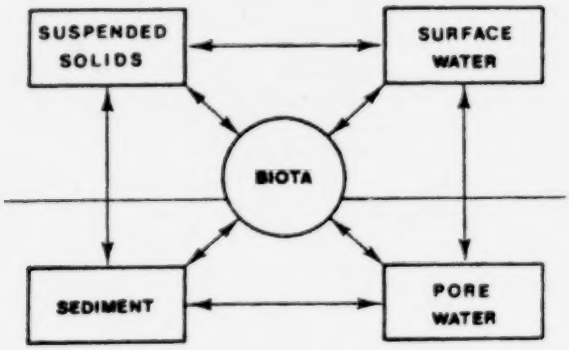

Fig. 1. Compartments and their interrelationships in the sediment-water system.

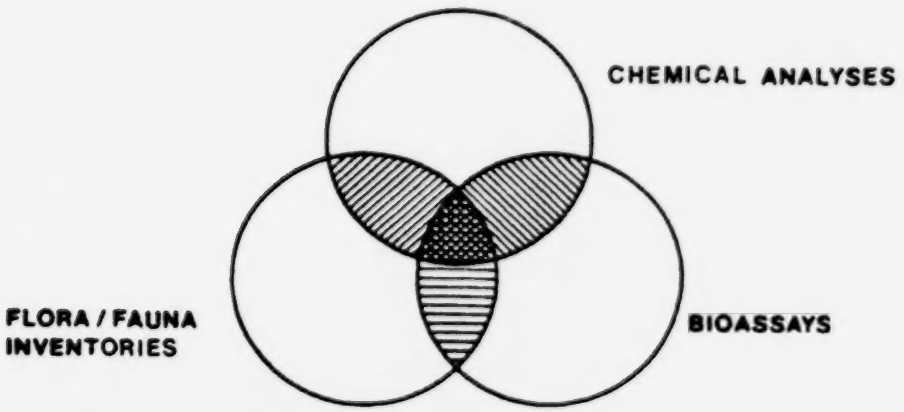

Fig. 2. Conceptual model of the impact assessment of contaminated sediments (after Chapman 1986).

To investigate whether the complementary tests with Chironomids reveal relevant information in addition to the results obtained with standard test organisms like e.g. Daphnids, a research program was started in which sediments from several locations were screened chemically, and biologically in several types of (semi)static bioassays. Additional experiments were carried out with *Daphnia magna* and *Chironomus riparius*, using a water-recirculating test system and using artificially contaminated reference sediments. Emphasis was laid upon the distinction between sediment- and water-concentration based effects.

MATERIALS AND METHODS

Sediments and chemical analyses

At 42 locations in the sedimentation area of the river Rhine the upper sediment layer was sampled using a Van Veen grab. The sediments were all stored wet at 5°C and handled according to standard laboratory practice (Mass and van de Guchte 1987). Chemical analyses were performed for the compounds listed in Table 1, using methods recommended by the American Public Health Association (1980) and the U.S. Environmental Protection Agency (1979). Contaminant concentrations were corrected for lutum and organic carbon content as present in a defined Dutch standard reference sediment. The sediments were classified using the Dutch interim procedure for judging the environmental contamination of freshwater and dredged sediments as indicated in Table 1 (van der Kooy 1987).

For compound specific studies, a sediment sampled in an undisturbed lake (Oostvaardersplassen) was used. Characteristics are given in Table 2. Compounds used (Cd, Hg, Dieldrin) were analytical grade and analyzed following previously mentioned procedures. Specific attention was given to the presence of several salts and ammonia (see Table 3).

Test procedures

Toxicity associated with the contaminated sediments was assessed by monitoring potential effects on several aquatic organisms exposed to (1) the extracted pore waters, and (2) the sediments in sediment-water systems (Table 4).

Chlorella, *Daphnia* and *Brachydanio* are routinely reared in our laboratory, for *Chironomous* a new culture was established (van de Guchte and Grootelaar, in prep.). *Photobacterium* and *Salmo* were obtained from Beckman Inc. and a fish hatchery at Vaassen (NL), respectively.

Pore water bioassays: Pore water was obtained by centrifugation of a sediment subsamples during 20 min at 6000 rpm, followed by aeration to bring initial oxygen content above 5 mg/L. Dilutions were made with a standard medium derived from Alabaster and Abram (1965); pH 8.2±0.1 and hardness 250 mg/L as $CaCO_3$.

Standard aquatic toxicology practice was used in performing the duplicate or triplicate test series. The tests were carried out at 20°C. All tests were run in glass vessels of appropriate size, depending on the test species studied. The short term bioassays (up to 96 h) were static tests, in which the animals were not fed. The test medium in the ELS-bioassays with *Brachydanio rerio* were renewed once after 96 h.

Table 1. Recommended values for the classification of freshwater and dredged sediments.

Parameter		Unit*	Recommended values		
			target-	directive-	limit-
Heavy metals					
Arsenic	-(As)	mg/kg	35	45	100
Cadmium	-(Cd)	"	1.4	7.5	30
Chromium	-(Cr)	"	115	155	600
Copper	-(Cu)	"	35	90	400
Nickel	-(Ni)	"	40	45	100
Lead	-(Pb)	"	70	160	700
Zinc	-(Zn)	"	300	1000	2500
Mercury	-(Hg)	"	0.5	1.6	15
Mineral oil		mg/kg	500	3000	5000
EOCL		"	5.5	7	20
Polycyclic Aromatic Hydrocarbons (PAH)					
Fluoranthene	-(Flu)	µg/kg	1200	2000	3500
Benzo(a)fluoranthene	-(B(a)F)	"	550	750	3500
Benzo(k)fluoranthene	-(B(k)F)	"	550	750	3500
Benzo(a)pyrene	-(B(a)P)	"	200	750	3500
Benzo(g,h,i)perylene	-(B(ghi)P)	"	200	750	3500
Indeno(1,2,3-cd)pyrene	-(IP)	"	200	750	3500
Σ of PAH - 6 of Borneff		"	2300	4600	17000
Other PAH - total		"	200	750	3500
Organohalogen compounds					
PCB-IUPAC # 28, 52, 101,138, 153, 180 - individual		µg/kg	4	30	100
Σ of 6 PCB		"	20	150	400
Individual (excl. PCB)		"	2.5	15	500
Σ of 10 individuals (excl. PCB)		"	20	100	2500
		Class:	I	II III	IV

* Units based on µg or mg/kg dry-weight for a reference sediment.

Sediment bioassays: Sediment subsamples were vigorously mixed with the standard A & A test medium for 1 d in a 1:4 volume-volume ratio and allowed to settle down in the glass test vessels for at least 3 d. In the trout ELS-bioassays a 20% A & A medium was used. If necessary, the water-phase was gently aerated prior to the start of the short term tests, or continuously in the long term tests with trout eggs.

In the ELS sediment bioassays the eggs were placed on a stainless steel screen just above the sediment in such a way, that the eggs made contact with the sediment but could not sink away.

The test medium in the short term bioassays was not renewed. The trout ELS media, including the sediment, were renewed once a week. The latter was carried out at 10°C, the other tests at 20°C. All assays were run in duplicate or triplicate. During the experiments the animals were not fed.

Table 2. Characteristics of the experimental reference sediment from Oostvaardersplassen.

Dry weight:	36%	As:	16.7 mg/Kg	Ni:	22.0 mg/kg
Organic carbon:	4%	Cd:	0.8 mg/kg	Pb:	48.0 mg/kg
Grain size:	<188 μm - 100%	Cr:	43.0 mg/kg	Zn:	169.0 mg/kg
	<16 μm - 68%	Cu:	18.0 mg/kg	Hg:	0.3 mg/kg
	<2 μm - 4%	Oil:	110.0 mg/kg	EOCl:	0.1 mg/kg
Total N (Kj):	4.0 mg/g dw	Σ of 6 PAH's:	6300 μg/kg		
Total P (P_2O_5):	0.9 mg/g dw	Σ of 6 PCB's:	9 μg/kg		
Fe:	28.8 mg/g dw				
Mn:	2.4 mg/g dw	Other organohalogen compounds (33): ND			

Additional experiments: With Daphnids acute and chronic studies on survival and reproduction were, for some sediment samples, also carried out in a water-recycling sediment-water test system as described by Prater and Anderson (1977).

(semi)static *Daphnia* experiments were performed with reference sediments and their pore waters, which were artificially contaminated with heavy metals. Stock solutions of the individual compounds, Cd and Hg respectively (as chlorides), were added to a slurry of sediment in standard A & A water and mixed for 24 h, after which settlement of the sediment could take place. After 3 d the bioassays started.

Acute and chronic studies on survival and larval development of *C. riparius* were conducted using several life stages and using reference sediments artificially contaminated with Dieldrin. Stock solutions were prepared in acetone. Further methods are comparable to those mentioned before.

Table 3. Additional variables measured in pore waters.

Conductivity (mS/m)	NH_4^+ (mg/L)	HCO_3^- (mg/L)	Na (mg/L)
pH	NO_3^- "	SO_4^{-2} "	K "
Oxygen (mg/L)	NO_2^- "	Mg "	Ca "

RESULTS

Sediment assessments

The classification of the sediments on the basis of chemical analyses and the results of the pore water and sediment bioassays are compared in Table 5. It is obvious that in the sediment bioassays only the higher classified sediments cause some toxic effects. The sampled pore waters generally showed effects that were at least similar, but often stronger than the corresponding sediments.

TABLE 4: Bioassays.

	pore water	sed / wat	duration	criteria	method
Photobacterium phosphoreum	A		15 min	luminescence inhibition	Bulich et al., 1981.
Chlorella pyrenoïdosa	A		96 h	growth inhibition	NEN 6506, 1980.
Daphnia magna	A !	A , C	48 h,21d	survival, reproduction	NEN 6501/6502, 1980.
Chironomus riparius	$A^{2,4}$	A^{4} $!^{2}$	96 h	survival 2^{nd}, 4^{th} instar	v.d.Guchte et al., in prep.
Brachydanio rerio	ELS	ELS	7 d	survival, anomalies	v.Leeuwen et al., in prep.
Salmo gairdneri		ELS	60 d	survival, growth, anomalies	v.Leeuwen et al., 1985.

A=acute, C=chronic, ELS=early life stage
! : chronic tests in preparation

TABLE 5: Sediment chemistry (classification) and bioassay results in pore water and in sediment - water testsystems.

sediment sample	class	NH_3	salt	pore water							sediment-water					
				Photob.	Chlor.	Daphnia	Chironomus L_2	Chironomus L_4	Brachy.		Daphnia A	Daphnia C	Chironomus L_4	Brachy.	Salmo S	Salmo G
1- 3	I-II	.	.	-	-	-	-	-	-		-	-	-	-	-	-
4	II	o	.	-	-	+	+	+	+		-	-	-	-	-	-
5-14	III	.	.	-	-	-	$+^{1}$	-	-		-	-	-	-	-	$+^{1}$
15-18	III	∅	.	-	-	+	ND	-	+		-	-	-	-	-	$+^{1}$
19-25	III	o	.	-	-	+	ND	+	+		-	-	-	-	+	+
26-32	III	∅	o	-	+	+	+	+	+		-	-	-	+	+	-
33-36	III-IV	∅	o	-	+	+	+	+	+	$+^{a}$	-	-	-	+	+	+
37-39	IV	.	ND	-	ND	+	ND	ND	+		-	ND	$+^{b}$	+	ND	
40-42	IV	.	.	-	ND	+	ND	+	ND		+	+	+	-	+	-

- = no significant effect, + = significant effect, 1 = only one sample showing effect, ND = no data, o = values indicating possible cause of effects, ∅ = idem, not significant, . = values acceptable, a = anomalies, b = behaviour.

As can be shown from Table 5 in more than half of the bioassays the recorded effects might have been caused by high ammonia or high salt contents respectively. It was therefore assumed that 2-5% of the measured NH_4^+ concentration could have been present in the toxic NH_3^- form, which causes significant effects at the lower ppm range (>2 mg/L). Salt contents, though also measured individually, were thought to be deleterious when total conductivity of the pore water exceeded 600 mS/m.

In the chronic *Daphnia* tests in sediment-water systems, often a higher reproduction as compared to the control was observed. A good food source or a hormesis phenomenon might play a role here.

Additional experiments

The water-recirculating test system revealed no information on *Daphnia* toxicity different from that obtained in the (semi)static short- and long-term experiments with these organisms. Therefore these results are not listed separately.

In the bioassays with the artificially contaminated sediments (1200 mg/kg dw Cd and 60 mg/kg Hg resp.) the metal concentrations in the overlaying water was about 0.01-0.1% of the sediment concentration. With the Hg loaded sediment no significant effects were observed on *Daphnia* survival. The Cd enriched sediment caused a 40% mortality within 48 h only when a 1:1 sediment-water ratio was used. Effects in the pore water were much more evident (Table 6).

The results of the additional experiments with *C. riparius* in Dieldrin contaminated sediments are presented in Fig. 3, a marked effect on the larval development is significant at concentrations of 0.11 mg/Kg. Some information on the variation in sensitivity between different developmental stages can be suggested from Table 7, the 2nd larval instar being the most susceptible to Dieldrin.

Table 6. Results of short term *Daphnia* bioassays with naturally and artificially Cd- and Hg-contaminated sediments and pore waters.

	Contaminants (mg/kg dw)		Survival (%)			
	Cd	Hg	sed[1] wat 1:4	sed wat 1:1	pore water 50%	pore water
Reference sediment	0.8	0.3	100	100	100	100
Iden, Cd enriched	1200		90	40	45	20
Iden, Hg enriched		60	95	95	100	80
Dredged sediment (Cu:24; Cr:23; Pb:11; Zn:87)	25	0.6	100	80	70	85
Dredged sediment (Cu:134; Cr:180; Pb:470; Zn:66)	42	0.7	10	0	10	0

[1] Test system with sediment : water ratio =1:4

Table 7. Susceptibility of *C. riparius* life stages to Dieldrin.

Test	Stage	Nominal conc.
96 h LC50	Egg	>100 µg/L
"	2^{nd} larval instar	5.2 µg/L
"	3^{rd} larval instar	12.6 µg/L
"	4^{th} larval instar	17.9 µg/L
$NOEC_{23}$ d	Development egg-L_4	0.1 µg/L

DISCUSSION

Only in the last few years is there a growing awareness of the possible impacts of polluted sediments on living biota (Chapman 1986; LeBlanc et al. 1985). Because chemical analyses alone do not provide information on the bioavailability of contaminants present, attention is now focussed on the suitability of bioassay procedures in judging sediment samples for their toxic properties.

Among the procedures that are recommended are those that incorporate the sediment in a sediment-water test system for reasons outlined above (see Fig. 1). In the research presented here, however, the sediment-water bioassays did not reveal more information on sediment toxicity than did the bioassays with the sampled pore waters (Table 5). Several reasons might account for this. After the vigorous mixing procedure humic substances could have dissolved from the sediment. This will reduce the bioavailable fraction. During the time sediment particles subsequently settle down, they still act as a 'sink' for dissolved pollutants. Next to this there simply might be introduced a dilution of the pore water concentrations of those chemicals, that desorb only slowly, or do not absorb to the sediment at all. The results of the assays with different sediment-water ratios (Table 6) seem to be indicative for these reasons. Therefore it is plausible that for a first, cost-effective screening procedure one can rely upon short-cut bioassay methods using sampled pore waters.

It should be stressed that before any bioassay is carried out, analytical data must be available on the presence of major elements in the sediment. Apparently an open door, but indicative for common practice in sediment research. In the greater part of the sediment tested, NH_3^- and salt contents exceeded the required species specific test conditions (Table 5), thereby masking the potential effects of the other toxicants. Especially in bioassays with pore waters from harbour sediments in the Rhine sedimentation area, salt water species are recommended as test organisms.

From the results presented in Table 5 it can also be concluded, though somewhat prematurely, that the Dutch interim classification scheme serves quite well the regulatory purposes (i.e. priority setting) of environmental management strategies dealing with polluted sediment locations; only in the case of Class IV sediment-types significant effects, not due to NH_3 or salt content, could be observed in the bioassays performed. However, long-term experiments have been conducted to accurately assess the potential long term environmental impact of Class III sediments.

The water-recirculating test system give no additional information on sediment toxicity as compared to the (semi)static sediment-water bioassays, probably because of the same two reasons as mentioned above. Because this bioassay only seems to

distinguish very polluted sediments, the relative complex test system is not recommended in a first screening of sediments.

Published literature data on the toxicity and bioaccumulation of sediment contaminants are not identical in indicating the main route of uptake by the test organisms; bioconcentration via the water phase and biomagnification via the ingestion of contaminated food or sediment particles are mentioned both (e.g. Landrum and Acavia 1983; Nebeker et al. 1986). The results of the additional experiments with Daphnids and Chironomids do support the first statement on bioconcentration.

The results of the bioassay with *D. magna* and Cd- and Hg- enriched sediment (Table 6) can be explained by assuming that toxicity is caused only by the dissolved fraction of the contaminants present. This agrees well with the finding of Nebeker et al. (1986). In the dredged sediment bioassays calculated free ion concentrations for Cd and Hg were much lower. Observed effects must be caused by other contaminants present, probably also chemicals not looked for. This favors the use of bioassays in environmental impact assessments.

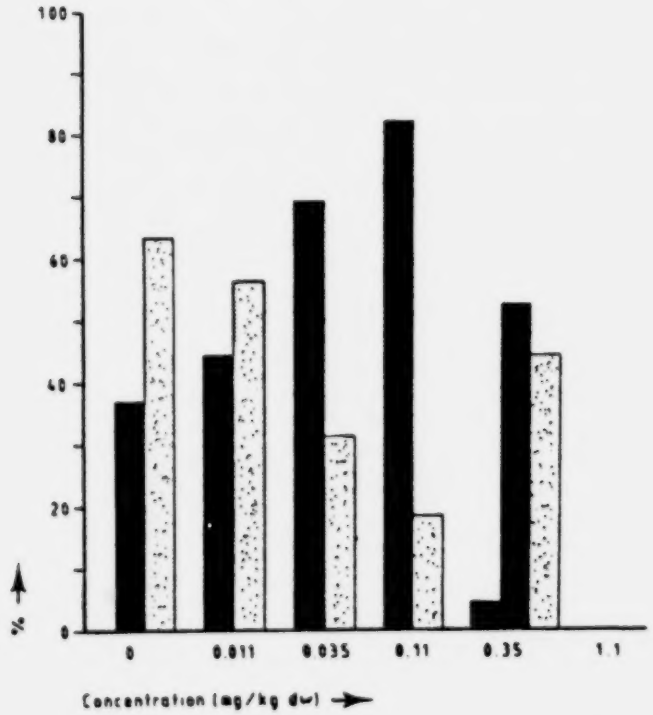

Fig. 3. Percentage of different larval instars of *C. riparius* after 16 d exposure (started with 2nd instar) to various Dieldrin concentrations in sediment. ■ 2nd instar ▩ 3rd instar ▭ 4th instar

A significant effect on larval development of *C. riparius* was observed in the Dieldrin contaminated sediment at a concentration of 0.11 mg/kg dw (Fig. 3). Using the equilibrium partitioning theory as described by Ziegenfuss et al. (1986) a pore water concentration of approximately 0.1 µg/L can be calculated. Relating this effect level to the reported NOEC value for a water-only system (Table 7), the hypothesis, that the main route of contaminant uptake, also from sediment inhabiting species, is one via the (pore)water, is again supported. Therefore it can be concluded that short-cut bioassays using pore water samples are suited to screen the potential impacts of polluted sediments, also when benthic organisms are involved.

Although it can be argued that the standard set of test species is then well established to serve the objectives of this sediment impact assessment, ecotoxicological data on indigenous species like Chironomidae should be generated, firstly to confirm this statement, and secondly, to make it possible to relate laboratory bioassay data to *in situ* macrofauna inventories. In the development of standard tests with *Chironomus sp.* attention should be focussed on the use of sensitive life stages (2^{nd} larval instar) in medium term (10 d) bioassays. Defining appropriate test conditions is a prerequisite.

ACKNOWLEDGMENTS

The ecotoxicological research on aquatic sediments presented here were performed by Albert Espeldoorn, Liesbeth Grootelaar, Kees van de Guchte, Wiely Luttmer, Hannie Maas and Gerrit Niebeek.

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HEPATIC CATALASE ACTIVITY OF METAL-EXPOSED TROUT: A TEST OF THE METALLOTHIONEIN 'SPILL-OVER' HYPOTHESIS

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ABSTRACT

The induction of metal-binding proteins in tissues of metal-exposed organisms is thought to represent metabolic detoxication. Metals complexed by metallothionein, a low-molecular weight, sulfhydryl-rich metal-binding protein, include Cd, Cu, Hg and Zn. The 'spill-over' hypothesis states that exposures to these metals are not toxic when the rate of induction of metallothionein and binding of metals exceeds the rate of metal accumulation. At toxic exposures, some metal remains unbound by metallothionein and free to bind to higher molecular weight proteins. Enzymes inhibited by these metals give rise to symptoms of toxicity. We have developed a reliable assay for measuring the activity of catalase, a metal-sensitive enzyme, in livers of fish. Optimum conditions for sampling, sample storage, and assay of enzyme activity have been described, as well as the sensitivity of the enzyme to metals *in vitro*. Exposure of fish to waterborne Cd, Cu, Hg and Zn for 21 d causes an inhibition of enzyme activity *in vivo* at concentrations that are chronically toxic. The association of enzyme inhibition with induction of metallothionein and distribution of metals in sub-cellular fractions will be described.

RÉSUMÉ

On pense que l'induction de protéines qui se fixent à des métaux dans les tissus d'organismes exposés à des métaux, représente une détoxication métabolique. Le Cd, le Cu, le Hg et le Zn sont des métaux complexés par la métallothionéine, une protéine à faible PM riche en sulfhydryle et qui se combine aux métaux. L'hypothese du "spill-over" suppose que l'exposition à ces métaux n'a pas d'effets toxiques tant que le taux d'induction de la métallothionéine et que la combinaison aux métaux dépasse le taux d'accumulation de ces derniers. Á des niveaux toxiques d'exposition, certains métaux échappent en partie à la métallothionéine et sont libres de se combiner à des protéines de PM supérieur. Les enzymes inhibées par ces métaux sont à l'origine des symptômes de toxicité. Nous avons mis au point un essai fiable pour mesurer l'activité de la catalase, une enzyme sensible aux métaux, dans le foie des poissons. Les conditions optimales d'échantillonnage, de conservation des échantillons et de mesure de l'activité enzymatique ont été décrites, ainsi que la sensibilité de l'enzyme aux métaux *in vitro*. L'exposition de poissons à du Cd, du Cu, du Hg et du Zn dans l'eau pendent 21 jours provoque une inhibition de l'activité enzymatique *in vivo* à des concentrations toxiques sur le plan chronique. La combinaison de l'inhibition enzymatique avec l'induction de la métallothionéine ainsi que la distribution des métaux dans les fractions de niveau sub-cellulaire seront décrites.

LOCOMOTOR ACTIVITY TESTS: METHODS AND APPLICATIONS

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ABSTRACT

Changes in locomotor activity have long been used in pharmacological and toxicological assay to quantify effects of drugs and toxins on mammals. Levels and temporal (diel and seasonal) patterns of locomotor activity are also basic responses reflecting adaptation of motile organisms to natural spatial and temporal variations of environmental conditions, e.g. temperature, oxygen, photoperiod. Consequently, changes in activity levels and patterns are being used to determine effects of environmental stressors. Referring in part to our own efforts and results, new development in two major methodological areas are described which appear to overcome some particular difficulties in work with aquatic species. The light beam interruption principle, most popular in work with terrestrial animals, has been replaced with ultrasonic beam techniques which allow continuous and automatic response quantification over day and night, in lab and field, in clear and turbid waters. Recent advances in computerized automated video image analysis in combination with low-light sensitive 'dry' or underwater video cameras are opening up highly efficient and increasingly affordable approaches. These novel methods and their applications are reviewed and discussed.

RÉSUMÉ

Les variations de l'activité locomotrice ont longtemps servi dans les essais pharmacologiques et toxicologiques à la quantification des effets des médicaments et des toxines sur les mammifères. Les niveaux et les rhthmes (quotidien et saisonnier) d'activité locomotrice constituent également des réactions fondamentales qui montrent l'adaptation d'organismes motiles à des variations spatiales et temporelles de conditions du milieu, p.ex., la température, l'oxygène, la photopériode. Par conséquent, les variations dans les niveau et les rythmes d'activité servent à déterminer les effets de stresseurs écologiques. Á la lumière de nos propres recherches et des résultats obtenus, nous décrivons les progrès dans deux grands domaines méthodologiques qui semblent surmonter certaines difficultés particulières au travail avec des espèces aquatiques. Le principe d'interruption d'un faisceau lumineux, formule la plus populaire pour l'expérimentation avec des animaux terrestres, a été remplacé par des techniques fondées sur l'utilisation de faisceaux, d'ultrasons qui rendent possible la quantification continuelle et automatique des réactions, de jour comme de nuiter au laboratoire comme sur le terrain, en eau trouble comme en eau limpide. Des progrès récents dans l'analyse informatisée et automatique des images vidéo, en combinaison avec l'utilisation de caméras vidéo sous-marines efficaces sous faible éclairement, ouvrent la voie à des méthodes très efficaces et de plus écononomiques. Ces méthodes nouvelles ainsi que leurs applications sont analysées.

PREPARATION AND OPERATION OF A MOBILE AQUATIC TOXICITY RESEARCH UNIT

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ABSTRACT

A mobile bioassay laboratory was restored and operated under controlled conditions and field conditions. Ninety-six h static bioassays were conducted on industrial effluents to determine the reliability of the trailer, the effects of ultraviolet exposure, aeration, and low temperature storage of cyanide components of gold-mine effluents, and the effects of dilution water hardness.

Split-sample tests between the mobile unit and the laboratory at Lakehead University indicated that the mobile laboratory can be operated reliably in the field. Effluent treatments had no significant effect on overall toxicity, but changes in the cyanide components may have been obscured by toxicity due to high metal concentrations. The effect of dilution water hardness could not be determined because of water supply problems.

RÉSUMÉ

Un laboratoire mobile pour des essais biologiques a été remis en ordre et exploité sous des conditions contrôlées et sur le terrain. Des essais biologiques statiques de quatre-vingt seize heures ont été faits avec des effluents industriels afin de déterminer la fiabilité de la remorque, les effets de l'exposition à l'ultra-violet, l'aération et le stockage à basse température des fractions cyanurées d'effluents de mines d'or ainsi que les effets de la dureté de l'eau de dilution.

Des tests faits sur de mêmes échantillons dans l'unité mobile et au laboratoire de l'université Lakehead ont indiqué que le laboratorie mobile peut être exploité sans problème sur le terrain. Les traitements appliqués aux effluents n'avaient pas d'effet spécial sur la toxicité globale, mais des modifications de la fraction cyanurée ont pu être obscuries par la toxicité attribuable à la concentration élevée en métaux lourds. L'effet de la dureté de l'eau de dilution n'a pu être déterminé par suite de problèmes d'alimentation en eau.

EXTENDED ABSTRACT

The 96 h static bioassay with rainbow trout (*Salmo gairdneri*) is the standard biological test for evaluating the toxicity of industrial effluents in Ontario. Regulatory monitoring of these effluents must be carried out in an established laboratory under stable conditions, but under some circumstances the on-site testing of effluents would be desirable. On-site testing would allow evaluation of toxicity with the actual receiving water, and reduce the usual delay between sampling and testing. In-depth studies could also be carried out to examine simulated treatment processes or the toxicity of intermediate streams in the industrial or effluent treatment process, without the excessive transportation costs or demand on facilities if these projects were carried out in a central laboratory.

Construction of permanent facilities at every site where such studies might be undertaken would not be economically feasible, but the relocation and operation of a mobile bioassay laboratory could be a viable alternative. A mobile unit must contain all of the components of a permanent laboratory: fish-holding facilities; test facilities; and a chemistry bench, and should have independent supplies of water and compressed air. Experience has shown that the fulfillment of these requirements does not guarantee reliable, reproducible test results; and so, prior to the use of the mobile laboratory in detailed research applications, a quality assurance/quality control (QA/QC) program must validate operation under field conditions.

In June, 1986, the Aquatic Toxicity Research Group (ATRG) at Lakehead University (LU) approached the Northwestern Regional office of the Ontario Ministry of the Environment (MOE) to determine the feasibility of refurbishing and operating a mobile bioassay laboratory, then located at the MOE laboratory in Thunder Bay. Initial funding was secured to move the trailer to a LU campus location where electricity, water, and compressed air were readily available. During the fall, 1986, physical systems were tested and restored as necessary by members of LU's Physical Plant, and a test program was carried out with duplicate tests being run simultaneously in the trailer and the LU laboratory. These tests produced virtually identical LC_{50}'s (Fig. 1) and demonstrated the reliability of trailer operation under controlled conditions with access to water and compressed air.

Additional funding was secured for field operation for a QA/QC program during the summer of 1987. The site chosen for operation was the Wastewater Treatment Plant in Marathon, Ontario. This location offered access to Lake Superior water, protection from vandalism, and the local availability of effluents from two pulp-and-paper mills, three gold-mines, and two base-metal mines. These effluents are sampled regularly by the MOE sample crew for testing at LU, so split-sample testing could be carried out without placing a heavier demand on the LU facilities or requiring additional sample transportation costs.

The trailer test capacity was greater than the number of split-samples which could be accommodated at LU, and rather than leave the system idle a secondary test program was undertaken to examine parameters potentially affecting the toxicity of effluents from the Hemlo gold-mines. These mines use a cyanide-based extraction process, and concern had been expressed that changes in effluent composition during transport to test facilities could give misleading toxicity results. Effluents from each mine were tested after one- and five-day exposures to ultra-violet radiation (UV), aeration, and low-temperature storage, to determine differences from effluents stored under "normal" conditions. Water hardness is known to affect effluent toxicity under certain conditions, and to fill the remaining test time, comparison tests were conducted using the normal dilution water from Lake Superior and water from the Marathon municipal groundwater supply (hardness about 50 and 150 mg/L as $CaCO_3$ respectively). Analyses of the effluents for free and total cyanide were performed by the MOE laboratory in Rexdale, and for metals by the MOE regional laboratory in Thunder Bay. All tests were conducted in accordance with the standard MOE protocol (Craig et al. 1983).

Initial delays in trailer set-up, involving electrical service and the development of an adequate pump configuration consumed one month of the four-month program. There was a long horizontal run and a high lift from the lake to the trailer, and because of the shallow depth the pump could not be protected from wave action. Water

supply continued to be an intermittent problem throughout the program, with two periods of prolonged interruption due to pump damage by storms, and the program was cut short by a storm which damaged the pump beyond repair. A second pump could not be installed in time to continue because of continuing bad weather, limiting the operational period to just over two months. Other systems in the trailer (compressed air, heating/cooling, lighting, chemical analysis, computerized data transmission) performed flawlessly. Sixty-four tests were completed, eight of which were split-sample tests with LU.

Two periods of high mortality in the fish-holding tanks were encountered (Fig. 2). These coincided with the pump interruptions noted above, during which the water temperature could not be controlled and water could not be replenished. For the rest of the program, fish mortality was within acceptable limits and not notably higher than in the LU laboratory.

Split-sample LC_{50}'s from the trailer and the LU lab were virtually identical and had overlapping confidence limits, with one exception (Fig. 3) where the trailer test yielded a significantly lower LC_{50} value. This test coincided with a water interruption and may have been influenced by increased temperatures in the test tank. Overall, these results confirm that tests conducted in the trailer are reliable and reproducible.

Effluent manipulation did not produce significant differences in toxicity (Figs. 4, 5, and 6) over the times tested, and differences in concentrations of free and total cyanide among treatments were slight and inconsistent among effluents (Table 1). Changes in toxicity of the cyanide components of effluents may also have been masked by the contribution to overall toxicity of the relatively high metal concentrations (Table 1). Interpretation of metal toxicity in these data is extremely difficult because of the complexity of metal mixture toxicity, and the variable pH and hardness of both the effluents themselves and individual concentration levels in the tests. A more rewarding area of study for this program might have been the effects of manipulation on metal concentrations, rather than cyanide.

Results of the dilution water hardness tests are ambiguous (Fig. 7). Two of the tests produced equivalent LC_{50}'s, but the other produced significantly different results. However, these tests were in progress when the pump was destroyed and the difference could be due to increased sensitivity of test fish due to the loss of temperature control. Increased sensitivity due to temperature could be unapparent in the other two tests because in one case the effluent was non-lethal, and in the other the toxicity of the effluent was high enough to mask the effects of temperature.

FIGURE 1. INITIAL SPLIT-SAMPLE TESTS

FIGURE 2. MORTALITY IN HOLDING

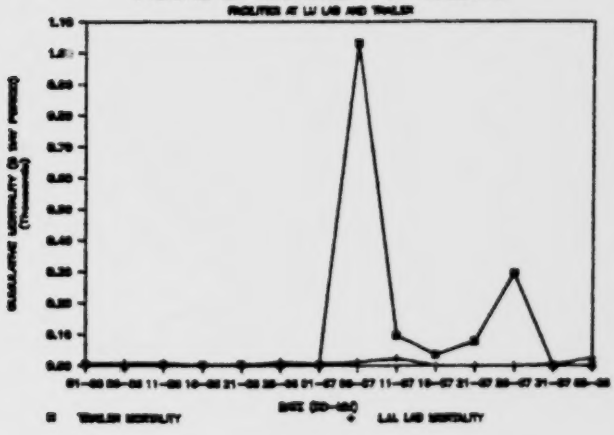

FIGURE 3. SPLIT-SAMPLE TESTING BETWEEN

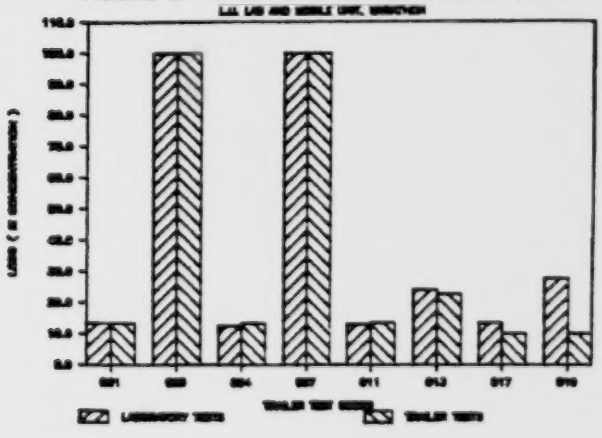

FIGURE 4. EFFECTS OF MANIPULATION ON

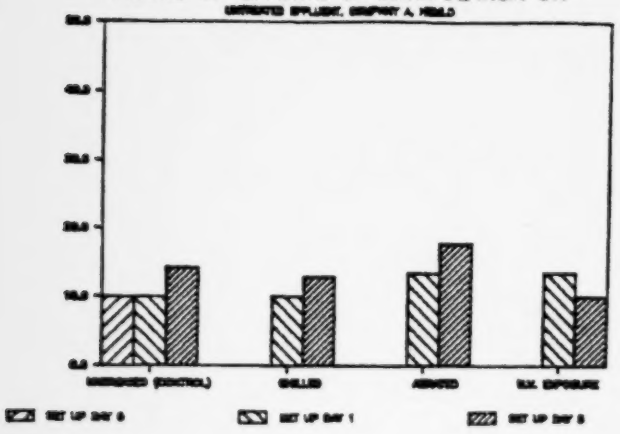

FIGURE 5. EFFECTS OF MANIPULATION ON

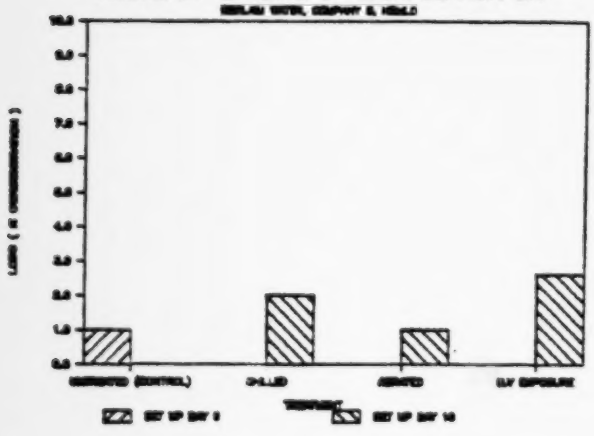

FIGURE 6. EFFECTS OF MANIPULATION ON

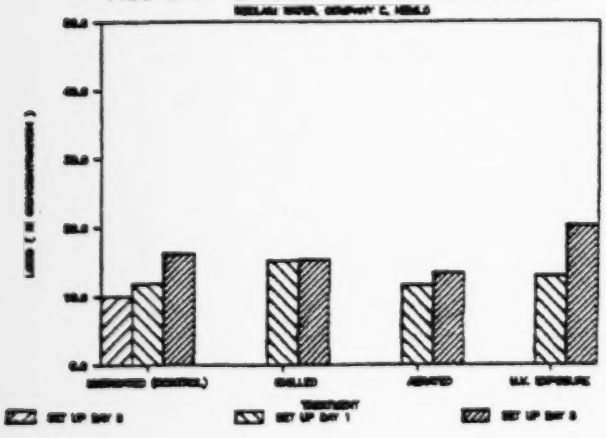

FIGURE 7. COMPARISON TESTS USING

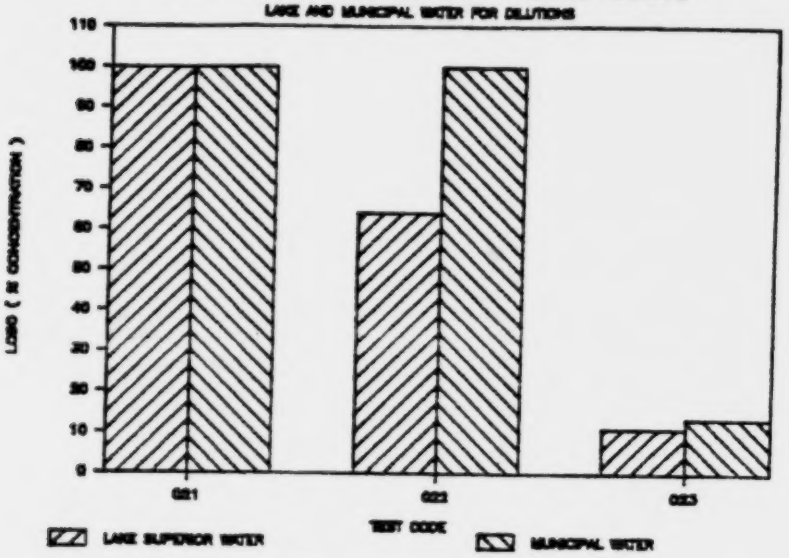

Table 1. Concentrations of free and total cyanide and selected metals before and after treatment.

Effluent	Treatment	Cyanide (mg/L) Free	Cyanide (mg/L) Total	Cu µg/L	Ni µg/L	Sb µ/L
A	Initial	<0.001[a]	0.056	330	90	14000
	Chilled	<0.001	0.047	340	75	14000
	Aerated	<0.001	0.041	300	90	14000
	UV exp.	<0.001	0.039	320	40	15000
B	Initial	8.80	9.60	4900	3900	-
	Chilled	10.30	9.30	4900	3900	-
	Aerated	8.50	7.70	5500	3900	-
	UV exp.	7.90	11.00	3900	2900	-
C	Initial	-	0.075	420	-	-
	Chilled	0.012	0.067	360	130	-
	Aerated	0.033	0.074	380	120	-
	UV exp.	0.006	0.054	390	150	-

[a] detection limit

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EFFECTS OF CHLORPYRIFOS ON MACROINVERTEBRATES IN LITTORAL ENCLOSURES

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ABSTRACT

Aquatic enclosures constructed in the littoral zone of a 0.72 ha pond in central Minnesota, were used to evaluate the impact of a mosquito larvicide chlorpyrifos on endemic macroinvertebrate communities. Chlorpyrifos was acutely toxic to several macroinvertebrates at a peak concentration of 17.3 ug/1. Midge (Chironomidae), mayfly (*Caenis sp.*) caddisfly (*Leptocerus americanus*) and damselfly (*Enallagma hageni*) populations were reduced or eliminated after application. However, oligochaete, snail, ostracod and clam populations increased in the treatment enclosure by the end of the experiment. Macroinvertebrate responses in the treatment enclosure were similar to previous field and laboratory bioassay data. Community similarity between the two enclosures remained high (>80%) before application but decreased to 55% by day 28. Community diversity also declined after exposure to chlorpyrifos. The littoral enclosure design proved to be an effective technique for evaluating primary (acute) effects of a pesticide on a natural pond ecosystem and should be readily adaptable to more complex experimental designs requiring replication of experimental units and longer-term studies.

RÉSUMÉ

Des enceintes aquatiques ont été installées dans la zone de rivage d'un étang de 0.72 ha dans la partie centrale du Minnesota; on voulait évaluer l'effet du larvicide pour moustiques chlorpyrifos sur les communautés endémiques de macroinvertébrés. Le chlorpyrifos s'est révélé être toxique façon aiguë pour plusieurs macroinvertébrés à une concentration de pointe de 17.3 µg/L. Les poplulations de cécidomyes (*Chironomidae*), de plécoptères (*Ceaenis* sp.) de phryganes (*Leptocerus americanus*) et de libellules (*Enallagma haqeni*) ont été réduites ou éliminées après l'application du larvicide. Cependant, les populations d'oligochètes, d'escargots, d'ostracodes et de bivalves avaient augment é dans l'enceinte de traitement à la fin de l'expérience. Les résultats obtenus dans l'enceinte avec les macroinvertébrés sont semblables aux résultats d'essais biologiques faits antérieurement sur le terrain et en laboratoire. La similitude des communautés réunies dans les deuxenceintes est restée élevée (>80%) avant l'application, mais elle est passeé à 55% au vingt huitième jour. La diversité des communautés s'est appauvrie après l'exposition au chlorpyrifos. Le principe de l'enceinte de rivage s'est révélé être une technique efficace d'évaluation des effets primaires (aiguës) d'un pesticide sur un écosystème naturel d'étang; il devrait être facilement adaptable à des protocoles d'expérience plus complexes nécessitant plusieurs groupes d'expérience et se prêter aux études à long terme.

INTRODUCTION

In recent years, aquatic toxicologists have advocated field validation research at the ecosystem level in an effort to evaluate the effects of potentially hazardous chemicals on natural aquatic systems (Cairns 1983; Kimball and Levin 1985). After evaluating the merits of many enclosure designs, a 'pilot' study was designed and evaluated using littoral enclosures which provide several advantages. Littoral enclosures are relatively inexpensive, can be replicated, incorporate natural undisturbed components of the ecosystem, include the littoral zone (important for understanding effects of pesticides on ponds dominated by the littoral zone), and are small enough to permit manipulation of biotic and abiotic conditions, yet large enough to support higher trophic levels.

Specific objectives of the macroinvertebrate study were to:

1) monitor changes in endemic macroinvertebrate populations and communities before and after pesticide treatment;
2) correlate laboratory LC_{50} data to the effects of chlorpyrifos on natural macroinvertebrate populations; and
3) evaluate the utility of the littoral enclosure design for macroinvertebrate studies.

METHODS

Two littoral enclosures were constructed in a natural pond in central Minnesota (Siefert *et al* 1987). On 16th May, 1985 chlorpyrifos was applied as an emulsifiable concentrate formulation to the water surface of one enclosure, reaching a peak concentration of 17.3 µg/L one hour after application. An untreated enclosure served as a reference.

Macroinvertebrate samples were collected with a 6 x 6 inch Eckman dredge from 11th May until 13th June 1985. Three random samples were collected from each enclosure on each sample data. Samples were preserved with a 10% formalin solution containing 100 mg/L Rose Bengal stain. After rinsing through sieves (425 µm), invertebrates were sorted, identified to genus or species, and enumerated.

A split-plot analysis of variance (Snedecor and Cochran 1967) and paired t-tests ($p > 0.05$) on log transformed counts were used to compare differences between treatment and reference enclosures. Community diversity and community similarity were described using Hurlbert's Probability of Interspecific Encounter, PIE, (Hurlbert, 1971) and Renkonen's Number (Wolda 1981).

RESULTS

Before pesticide application, the reference and treatment enclosure communities were dominated by species of oligochata, chironomidae (Diptera), ostracoda, *Caenis* sp. (Ephemeroptera), and *Leptocerus americanus* (Trichoptera). After application, oligochaetes dominated both communities, increasing in the treatment enclosure as the abundance of sensitive insect groups declined. Community diversity, PIE, was slightly higher in the treatment enclosure until 7 days after pesticide application (Figure 1). Community similarity between the two enclosures decreased from 81-89% before application to 55%, 28 days after application (Figure 1).

Total macroinvertebrate densities in the reference and treatment enclosures were similar before chlorpyrifos application shown by species diversity and percent similarity. Chironomids, mayflies (*Caenis* sp.) and other insects may be greatly reduced or eliminated in systems treated with the mosquito larvicide chlorpyrifos. The elimination of chironomids, important herbivores, predators and forage for fish (Gerking 1962; Macek *et al* 1972), could seriously disrupt the ecology of the system. Similarly, a reduction of grazing mayfly populations could allow filamentous algae blooms to occur (Crossland 1982). Enhanced algal production may have allowed the increase in omnivores and herbivores, including ostracod and snail populations. Although not conclusive, phytoplankton chlorophyll *a* was four times greater in the treatment enclosure on day 28.

Reported laboratory LC_{50} data were compared to weighted average chlorpyrifos concentrations calculated from field chemistry data (Table 1). The response of macroinvertebrate populations observed in the treatment enclosure indicates that comparisons of weighted concentrations with field responses were consistent with expectations derived from the laboratory data for all taxa.

Overall, the enclosure study revealed that chlorpyrifos can severely impact natural populations of macroinvertebrates based on the density and diversity reductions observed in the treatment enclosure. These impacts were consistent with reported laboratory and field response data for many macroinvertebrate populations. The littoral enclosure design proved to be an effective technique for evaluating primary effects of a pesticide on a natural pond ecosystem and should be readily adaptable to more complex experimental designs requiring replication of experimental units and longer-term studies.

ACKNOWLEDGEMENTS

We thank Sandra Beder-Miller for statistical advice and Steven Lozano and Frank Stay for reviewing this manuscript. We are also thankful for the advice and support of other members of our research group at the Environmental Research Laboratory-Duluth. This research was funded, in part, by the U.S. Environmental Protection Agency, the Computer Sciences Corporation and the Center for Lake Superior Environmental Studies, University of Wisconsin-Superior.

Table 1. Comparison of chlorpyrifos toxicity data to observed responses of treated macroinvertebrate populations in littoral enclosures in 1985.

	Hours			
	24	48	96	
	Time Weighted Field Concentration (µg/L)			
	8.58	6.84	3.80	
Organism	Laboratory[c] LC50 (µg/L)			Observed Field Response[b]
Diptera				
Chironomidae sp.	0.4-40.0	-	-	D
Chaoborus americanus	2.36[a]	-	-	D
Ephemeroptera sp.	0.9	0.4	-	D
Trichoptera sp.	-	0.87	-	D
Hemiptera				
Neoplea striola	2.42	-	1.22	D
Crustacea				
Hyalella azteca	-	<0.5	-	D
Ostracods sp.	-	-	>6.93	N
Gastropoda	-	-	>806	N
Oligochaeta sp.	-	-	1500	N

a LC_{50} at 18 hours.

b D = Decrease in abundance after application.
N = No acute effect, increase in abundance after application.

c Laboratory data from Marshall and Roberts, 1978; Mulla et al., 1979; U.S. EPA, 1986; Siefert et al., 1984.

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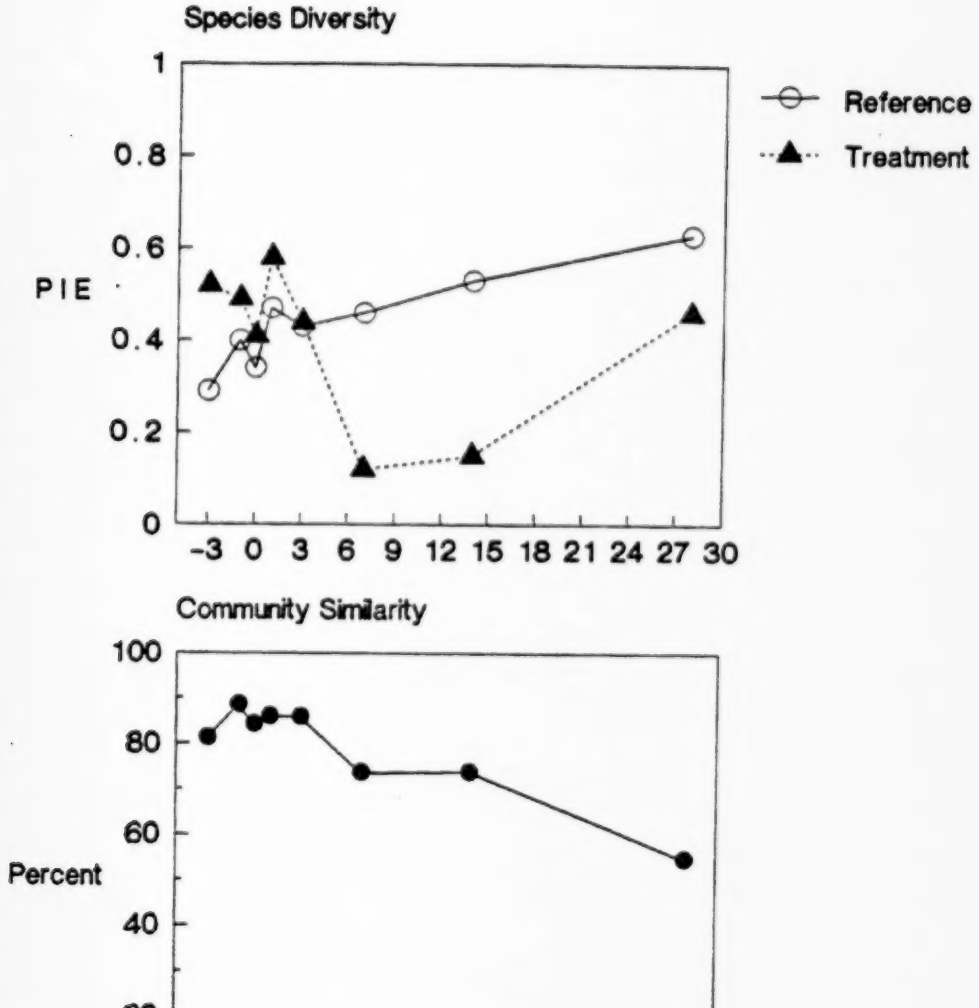

Figure 1. Species diversity and similarity of macroinvertebrate communities in reference and treatment littoral enclosures in 1985. (Day 0 denotes chlorpyrifos application)

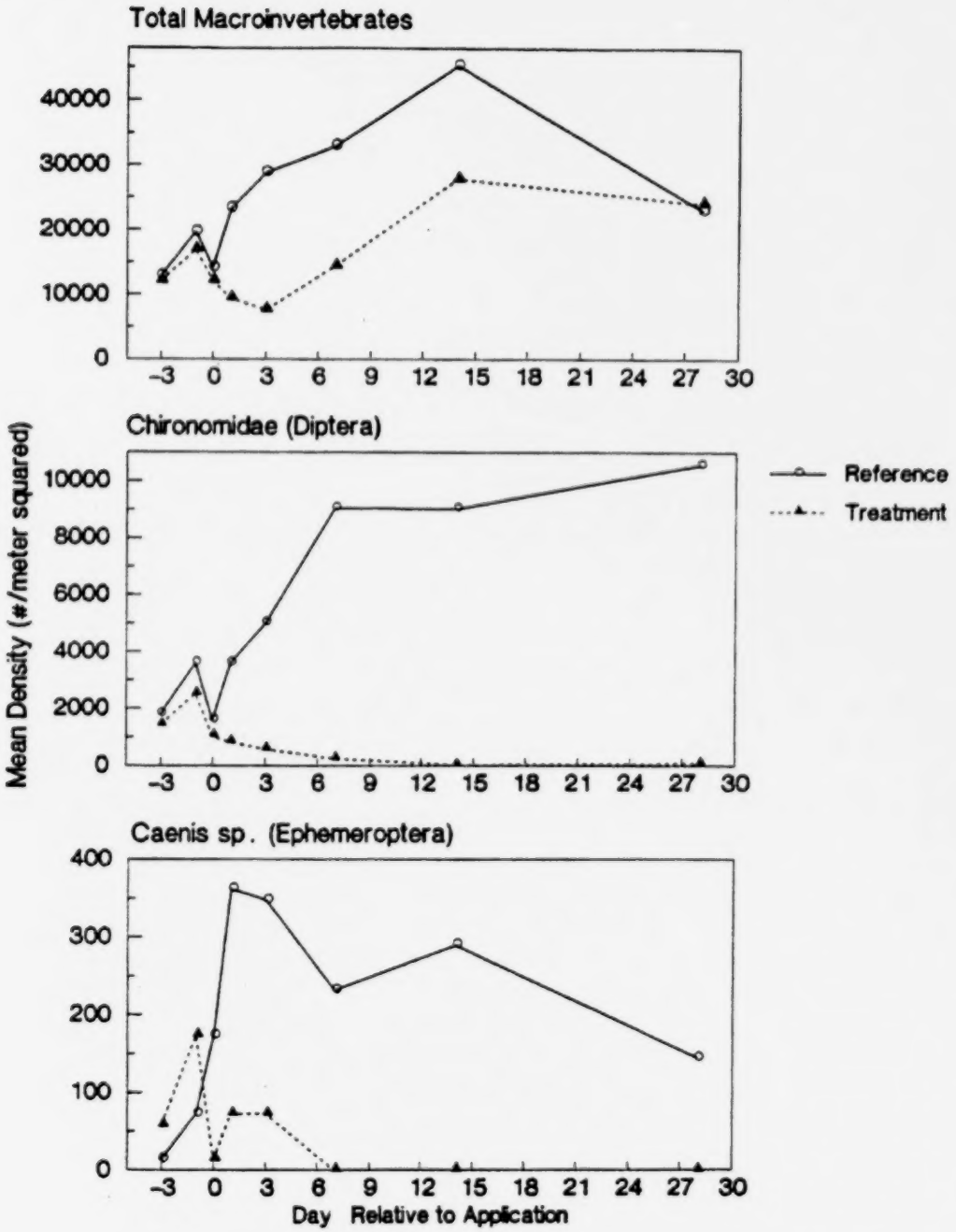

Figure 2. Macroinvertebrate populations in reference and treatment littoral enclosures in 1985.
(Day 0 denotes chlorpyrifos application)

THE POTENTIAL OF AVOIDANCE-PREFERENCE IN ESTABLISHING WATER QUALITY CRITERIA, BASED ON STUDIES OF SUBLETHALLY PRE-EXPOSED RAINBOW TROUT TO CR AND CU COMPOUNDS

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ABSTRACT

Scientists have recognized the potential of avoidance preference studies for evaluating sublethal effects of various toxicants for over 70 years. Unfortunately, the results of these studies were frequently unreliable due to incompatible designs, poor methodology and insufficient comparative studies to establish a standardized approach. A methodology has been developed (Anestis and Neufeld 1986) for conducting avoidance-preference studies using non-exposed as well as sublethally pre-exposed populations for 7-20 wk in order to establish the effect of previous exposure of the organisms on their behaviour.

Results obtained from two Cr and one Cu compound followed similar trends with the following general characteristics. (1) Fish demonstrated a preference for the toxicant at pre-exposure concentrations, demonstrating a recognition of a familiar environment. (2) Pre-exposed fish appeared more tolerant to the presence of the toxicant. Such tolerance increased with pre-exposure. (3) Avoidance threshold values increased linearly with increasing levels of pre-exposure. (4) In all cases a critical point of pre-exposure could be established, which was correlated to M.A.T.C. for the particular toxicant in use. (5) Avoidance threshold values were correlated to safe exposure levels. (6) Fish short-term recovery from toxicant pre-exposure depended on chemical characteristics of the ionic species. Based on such results avoidance mechanisms are proposed and the significance of avoidance-preference tests is demonstrated as a quick and efficient method to screen toxic agents and establish water quality standards.

RÉSUMÉ

Il y a soixante-dix que les chercheurs connaissent le potentiel des études des comportements d'évitement pour l'evaluation des effets sublétaux de différents toxiques. Il est regrettable que les résultats de ces études soient souvent peu utilisables en raison d'incompatibilités au niveau des méthodes, l'ume mauvaise méthodologie et des nombre insuffisant d'études comparatives qui auraient permis de normaliser cette méthodologie. Une telle méthodologie a été mise au point (Anestis et Neufeld (1986)) pour la tenue d'études du comportement d'evitement sur des populations non exposées ainsi que sur des populations sensibilisées au niveau sublétal pendant 7 à 20 semaines, cela afin d'etablir l'effet de la sensibilisation d'organismes sur le comportement.

Les résultats obtenus avec deux composés du chrome et un composé du cuivre s'apparentaient au profil général suivant. 1) les poissons montraient une préférence pour le toxique aux concentrations de sensibilisation, ce qui traduisait une reconnaissance d'un milieu familier. 2) les poissons sensibilisés paraissaient mieux tolérer le toxique. Cette tolérance était accure avec la sensibilisation. 3) les valeurs seuil du comportement d'évitement augmentaient linéairement avec l'augmentation des concentrations

durant la sensibilisation. 4) dans tous les cas, un point critique de sensibilisation pouvait être établi; celui-ci était en corrélation avec la concentration maximale acceptable du toxique utilisé. 5) les valeurs seuil du comportement d'évitement étaient en corrélation avec les niveaux d'exposition sans danger. 5) la récupération à court terme des poissons sensibilisés à des toxiques dépendait des caractéristiques chimiques de l'espèce ionique. Á la lumière de ce type de résultats, des mécanismes d'évitement sont proposés et il est établi que les tests mesurant les comportements d'évitement consituent une méthode rapide et efficace de sélection préliminaire d'agents toxiques et d'établissement de normes de la qualité de l'eau.

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L'ESSAI MICROPLAQUE DE LA TOXICITE L'ANALYSE FAITE A LA RONDE AVEC *SELENASTRUM CAPRICORNUTUM*

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RÉSUMÉ

Trois laboratoires d'écotoxicologie du Québec ont participé à un test comparatif inter-laboratoires, récemment publié, visant à évaluer l'efficacité d'un test peu coûteux de microtoxicité algaire. Les objectifs étaient : 1) du vérifier la fiabilité et al reproductibilité des résultats du test biologique, 2) d'optimiser l'éventuelle uniformité des méthodes. Trois séries d'expériences ont été faites avec six techniciens (deux de chacun des trois laboratoires) sur deux toxiques de référence (Cd^{2+} : $CdCl_2$ et phénol). Les variables étaient notamment la technique de culture des algues (série 1), la composition du milieu de croissance (série 2) et l'échange gazeux passif-actif durant l'incubation (série 3). Les observations essentielles et les conclusions qui se dégagent de cette étude inter-laboratoires seront présentées à l'atelier de travail.

ABSTRACT

Three Quebec-based ecotoxicological laboratories participated in an intercalibration exercise to assess the performance of a recently-published cost-efficient algal microtoxicity assay. The aims were (1) to verify the reliability and reproducibility of this bioassay, and (2) to optimize future procedural uniformity. Three test series were carried out with six operators (2 from each of the three labs) and two reference toxicants (Cd^{2+}:$CdCl_2$ and phenol). Variables included algal cultivation technique (series 1), growth medium composition (series 2), and passive/active gas exchange during incubation (series 3). Essential findings and conclusions stemming from this interlaboratory study will be presented at the Workshop.

INVESTIGATIONS ON THE TOXICOKINETICS OF CYANIDE IN JUVENILE RAINBOW TROUT (*SALMO GAIRDNERI*)

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ABSTRACT

The object of this research project was the toxicokinetics of cyanide and thiocyanate in relation to their possible effects on the thyroid gland of rainbow trout. Juvenile trout were exposed to sublethal concentrations of cyanide (0.01 mg/L HCN, 0.02 mg/L HCN) at different times of the year under laboratory conditions for periods up to 30 d.

We measured the bioaccumulation of cyanide (HCN) and thiocyanate (SCN) in the blood plasma of trout. The concentrations of plasma cyanide reached their maxima within the first 48 h of exposure and remained constant until the end of the experiment. Plasma concentrations in the summer were about 2 times higher than in the winter. The concentrations observed during summer, after 15 d of exposure, were of 3.05 μM HCN and 2.34 μM HCN for the highest and lowest exposure concentrations respectively. These values were 1.25 μM HCN and 0.66 μM HCN during winter. Intriguingly, fish showed a definite ability to bioconcentrate cyanide from the water which was also affected by the season. At the lowest exposure concentration the bioconcentration factor was of 6.3 during summer and of 1.79 during winter while for the highest concentration they were of 4.12 and 1.69 respectively. No dose-response relationships could be demonstrated between the plasma concentrations and the cyanide levels in the water.

Patterns of thiocyanate accumulation in the blood plasma of trout agreed with earlier works. Higher concentrations were observed in the summer than during winter; 31.92 μM SCN and 25.59 μM SCN for the lowest cyanide concentration and 51.92 μM SCN and 28.82 μM SCN for the highest on day 15. During winter maximum concentrations were established by day 15 and remained constant until the end of the experiment. No dose-response relationships were established between the plasma concentrations and the cyanide levels in the water.

The clearance of thiocyanate from plasma was also monitored for 15 d following a 30 d exposure period to cyanide. From 7 to 15 d were required for the complete elimination of thiocyanate.

The height of the epithelial cells of the trout thyroid follicles were also measured. After 15 d of cyanide exposure, during the summer, the highest exposure concentration (0.02 mg/L HCN) produced a 13% increase in their height.

RÉSUMÉ

Le présent rapport de recherche avait pour objectif la mesure de la cinétique toxique du cyanure et du thiocyanate en relation avec les effets possibles de ces substances sur la glande thyroïde de la truite arc-en-ciel. Des truites juvéniles ont été exposées à des concentrations sublétales de cyanure (0.01 mg/L HCN, 0.02 mg/L HCN) à

différentes périodes de l'année, en laboratoire, et sur des périodes pouvant atteindre 30 jours.

Nous avons mesuré la bioaccumulation du cyanure (HCN) et du thiocyanate (SCN) dans le plasma sanguin de la truite. Les teneurs en cyanure dans le plasma atteignaient un maximum dans les premières 48 h d'exposition et restaient constantes jusqu'à la fin de l'expérience. Durant l'été, elles étaient environ deux fois supérieures à ce qu'elles étaient durant l'hiver. Aprés 15 jours d'exposition, elles étaient de 3.05 μM HCN et de 2.34 μM HCN pour les concentrations l'exposition la plus élevée et la plus basse, respectivement. L'hiver, les valeurs passaient à 1.25 μM HCN et 0.66 μM HCN. Nous avons été étonnés de constater que les poissons parvenaient nettement à concentrer le cyanure dans ume mesure qui variait selon les saisons. A la concentration la plus basse d'exposition, le facteur de concentration atteignait 6.3 durant l'été et 1.79 durant l'hiver, alors qu'à la plus forte concentration, ce facteur passait à 4.12 et 1.69, respectivement. Aucun rapport dose-effet n'a pu être montré entre la concentration mesurée dans le plasma et la concentration en cyanure de l'eau.

Les modes d'accumulation du thiocyanate dans le plasma sanguin de la truite confirment les résultats de travaux antérieurs. L'été, on observait des concentrations supérieures à ce qu'elles étaient l'hiver : 31.92 μM SCN et 25.59 μM SCN pour la plus basse concentration en cyanure et 51.92 μM SCN et 28.82 μM SCN pour la plus forte concentration au jour 15. Durant l'hiver, la concentration maximale était obtenue au jour et restait constante jusqu'à la fin de l'expérience. Aucun rapport dose-effet n'a été établi entre la concentration dans le plasma et la concentration de cyanure dans l'eau.

La clearance du thiocyanate dans le plasma a également été étudiée pendent 15 jours après 30 jours d'exposition au cyanure. Il a fallu 7 à 15 jours pour éliminer totalement le thiocyanate.

La hauteur des cellules épithéliales des follicules thyroïdiens de la truite a également été mesurée. Aprés 15 jours d'exposition au cyanure, durant l'été, la plus forte concentration utilisée (0.02 mg/L HCN) avait produit une augmentation de hauteur de 13%.

MISE AU POINT D'INDICATEURS BIOCHIMIQUES ET CELLULAIRES DE LA QUALITE D'UN ENVIRONNEMENT MARIN

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RÉSUMÉ

Les rejets anthropogéniques dans les estuaires et dans le milieu marin en général sont un problème pour notre société. Toutefois, les effets directs ou combinés des polluants ainsi que les effets de stress environnementaux sur les individus et sur les populations peuvent être évalués à l'aide d'indicateurs précis du stress. Depuis trois ans, des indicateurs cellulaires et biochimiques de niveaux de pollution sous-léthale ont été élaborés dans nos laboratoires. Les stress environnementaux tels que la température, la salinité et autres, ainsi que les effets de concentrations sous-léthales de polluants peuvent être detectes par la variation de l'activité de la malate déshydrogénase dans le manteau et le muscle adducteur postérieur de la moule bleue, *Mytilus edulis* L. De plus, les activités de l'enzyme malique et de la glutamate déshydrogénase ont été choisies pour détecter les contaminations chroniques et aigués de la crevette *Pandaleus borealis* dans le fjord du Saguenay. Le stress occasionné par un déversement de pétrole a pu être évalué par un indicateur cellulaire général, l'étude de la stabilité de la membrane lysosomale, et par la mesure des réponses spécifiques à la présence de pétrole par l'étude de l'induction des activités de la NADH et de la NADPH cytochrome C réductases, des enzymes faisant partie du système MFO. Un apercu des méthodes utilisées, de leur utilité et des résultats obtenus à date sera présenté.

ABSTRACT

Our society has to deal with the impact of human activities on the marine environment. Direct or combined effects of pollutants and/or environmental stress on individuals or on populations can be predicted and evaluated by the development of accurate and precise indicators of stress. In the last three years, some cellular and biochemical indicators have been developed in our laboratories. Environmental stress (temperature, salinity and others) or sublethal effects of pollutants can be detected by the variation of some enzyme activities such as the malate dehydrogenase and pyruvate kinase, in mantle and posterior adductor muscle of the blue mussel, *Mytilus edulis* L. Furthermore, the activities of the malic enzyme and the glutamate dehydrogenase have been used for the detection of chronic or acute mercury contamination of the shrimp, *Pandaleus borealis* from the Saguenay fjord. Stress induced by an oil spill could be evaluated with a general cellular indicator, the lysosomal membrane fragility. Also, the induction of the MFO system, (NADH and NADPH cytochrome C reductase activities) a group of specific biochemical indicators of stress induced by oil has been evaluated. An overview of the methods used, their utility, and results will be presented.

INTRODUCTION

La société de nos jours doit se préoccuper de la qualité de l'environnement marin pour éviter que les rejets anthropogéniques ne détériorent de facon irréversible les

écosystèmes. Les différents gouvernements des pays riverains ont élaboré ces dernières années plusieurs programmes de vérification des sources de pollution en milieu marin pour leur permettre de mieux protéger leurs rives. Ces sources de pollution, potentiellement toxiques pour les organismes qui vivent dans ces écosystèmes peuvent être évaluées et classées selon leur degré de toxicité à l'aide de biotests qui permettent de déterminer les effets aigus des contaminants sur les processus vitaux d'un organisme et peuvent aussi évaluer les modifications irréversibles des processus biologiques, mais sans entraîner la mort des organismes. Une autre approche pour evaluer la qualité de l'environnement marin utilise des organismes qui ont une capacité de bioaccumulation proportionnelle à la concentration ambiante de polluant (Konasewich et al. 1986). Ces organismes sont plutôt des indicateurs de pollution sous-léthale qui aident à quantifier à l'aide d'organismes sentinelles le niveau biologique disponible de contaminants dans les écosystèmes aquatiques. Cependant, ces bioindicateurs donnent plutôt un apercu de la biodisponibilité du polluant plutôt que de l'abondance de celui-ci. L'organisme utilisé comme bioindicateur peut aussi réagir avec le contaminant, ce qui invalide la prémisse de départ pour un bon bioindicateur, qui est de réfléter les niveaux de contamination de l'environnement ambiant. La bioaccumulation peut être directement proportionnelle aux concentrations de polluant en contact avec les organismes tandis que des concentrations plus faibles conduisent à une régulation métabolique des polluants par l'organisme, possiblement via les voies de détoxification présentes chez la moule bleue.

Le choix d'un bioindicateur de pollution sous-léthale n'est donc pas simple car tel qu'indiqué par Phillips (1986):

- la capacité de bioaccumulation est fonction de la concentration de polluants dans l'environnement;
- la présence de régulations métaboliques fausse l'évaluation des quantités de polluants disponibles;
- la nature du contaminant et sa demie-vie influencent la bioaccumulation;
- la vitesse d'équilibration entre le contaminant et les tissus dépend de plusieurs facteurs ont l'état physiologique des individus;
- les organismes sessiles ou sédentaires habituellement choisis comme bioindicateurs et qui présentent une bioaccumulation élevée peuvent autant refléter une exposition aiguë près d'un effluent que l'exposition chronique au polluant.

Le choix d'un bioindicateur de pollution sous-léthale, nécessite donc des études préliminaires qui serviront à prouver sa capacité d'indicateur fidèle de la qualité d'un environnement aquatique et aussi qui serviront:

- à comprendre les processus de bioaccumulation dans cet organisme, en fonction du contaminant et de sa demi-vie;
- à spécifier les variations spatiales de la quantité de polluants et sa biodisponibilité;
- à spécifier les changements dans la biodisponibilité en fonction du temps à un ou plusieurs sites;
- à déterminer la présence de un ou plusieurs contaminants.

Conscients de ces contraintes, nous avons voulu verifier par l'étude de réponses cellulaires et biochimiques si, *Mytilus edulis*, ne pouvait pas nous procurer un bon indicateur de pollution sous-léthale, laquelle serait non-détectable par des analyses de bioaccumulation mais refléterait les régulations métaboliques de l'organisme face à un

contaminant, en évaluant les effets de toxicité sous-léthale de contaminants sous leur deux formes cellulaires, combinée ou de détoxification et sous forme libre et toxique dans le cytosol.
otre *objectifs général* est donc de calibrer les réponses générales et les relations spécifiques en fonction de la dose du contaminant, et d'établir les niveaux de toxicité relative de différents contaminants.

Nos *objectifs spécifiques* se formulent ainsi:

1. Etablir chez *M. edulis*, un indicateur biochimique de niveaux de pollution sous-léthale au méthylmercure par la mesure de differents paramètres cinétiques de la malate déshydrogenase, une enzyme-clé du métabolisme aérobique, par sa contribution au transport du malate et de la NADH, conduisant ainsi a la formation d'ATP dans la mitochondrie;

2. Evaluer le potentiel de trois enzymes chez la crevette nordique *Pandaleus borealis*, comme indicateurs d'effets du méthylmercure sur le métabolisme de cet organisme. Nous avons choisi; (a) l'enzyme malique qui est impliquée dans le métabolisme des graisses par la production de NADPH utilisee par la suite dans l'étape réductrice de la synthèse des lipides; (b) la glutamate déshydrogénase qui est située au carrefour du métabolisme des acides aminés, du cycle de Krebs et du systéme d'excrétion de l'azote et enfin; (c) la glutathione peroxydase qui joue un rôle de protection de la cellule contre les effets des radicaux libres et des hydroperoxides.

3. Intégrer les changements causés par les stress environnementaux au niveau de la structure cellulaire et de la biochimie (Bayne et al. 1985) par une méthode cytochimique qui mesure la fragilité de la membrane lysosomale (Moore 1976). Les lysosomes sont des organelles subcellulaires capables de cataboliser des composés cellulaires endogènes et de séquestre des substances xénobiotiques. Les lysosomes d'organismes non stressés sont imperméables à beaucoup de substrats et les hydrolases sont inactives. Différentes conditions de stress déstabilisent la membrane lysosomale, augmentent la pérméabilité de la membrane aux substrats, activent les enzymes hydrolytiques et libèrent les hydrolases dans le cytoplasme produisant des effets cataboliques à ce niveau (Bayne et al. 1985).

MATERIEL ET METHODES

Le protocole expérimental a été décrit antérieurement par Pelletier (1986) et Pellerin-Massicotte (1987). En résumé, le processus de contamination a été réalisé grâce à l'appareillage illustré à la Fig. 1. L'eau de mer est pompée dans les bassins contenant les moules (Fig. 2) à partir d'un bac a décantation tandis que les solutions-mères de sélénium (Na_2SO_3) et de méthylmercure [$(CH_3Hg)_3O$ OH] sont gardées dans des réservoirs de verre de 13 L (A,B,C,D) et mélangées ultérieurement avec de l'eau de mer à l'aide d'une pompe péristaltique. De fines particules de TiO_2, agissant comme transporteur inerte, ont été ajoutées aux solutions-méres pour garder les polluants en solution et réduire les pertes de mercure par adsorption sur la tubulure de plastique et les parois de verre. Les effluents contaminés sont par la suite filtrés sur un mélange de sable/charbon avant d'être rejetés dans le drain du laboratoire. La photopériode a été réglée à 12 h.

Des moules bleues ont été échantillonnées (3.5±0.3 cm) sur un substrat rocheux à Pointe Mitis (48°40'N, 68°02'W) le long du littoral de l'estuaire du St-Laurent. 150

mussels ont été placeés dans chaque bassin et gardees 24 h avant le début de l'expérience. Six bassins ont été utilisés et les moules ont été mises en présence des solutions suivantes pour une période de 29 jours.

1. Contrôles (eau de mer)
2. 0.3 µg/L méthylmercure
3. 3.0 µg/L méthylmercure
4. 125 µg/L sélénium
5. Sélénium + 0.3 µg/L méthylmercure
6. Sélénium + 3.0 µg/L méthylmercure

La température moyenne de l'eau de mer fut de 12±2°C et la salinité fut de 25.61±1.83 °/oo pendant le protocole expérimental. Les moules ont été nourries avec *Phaeodatylum tricornutum* et de l'eau de mer en continu pendant tout le protocole expérimental. 25 moules ont été prélevées aux jours 0, 1, 3, 7, 15, 22 et 29; la nourriture et le niveau de polluant a été maintenu constant grâce au remplacement des moules prélevées par d'autres moules mises dans les bassins dans un filet de Nytex^{R}. Les moules, les jours d'échantillonnage, ont été pesées, les manteaux et les muscles adducteurs postérieurs ont été disséqués, pesés et les sexes déterminés par l'examen microscopique de frottis du manteau. Les fractions cytosoliques et mitochondriales ont été obtenues par centrifugation differentielle des homogénats tisssulaires et gardés ultérieurement à -70°C avant les analyses enzymatiques. Les protéines totales solubles ont été mesurées à l'aide des réactifs achetés chez BIO-RAD et en utilisant de l'albumine bovine comme standard. L'activite dé la malate déshydrogénase dans les fractions cytosoliques a été réalisée en suivant l'oxydation de la NADH à 340 nm avec un spectrophotométre Perkin-Elmer Coleman 575. L'oxaloacetate a été utilisé comme substrat avec 5 µg de protéines par essai enzymatique, pour un volume total de 3.1 mL.

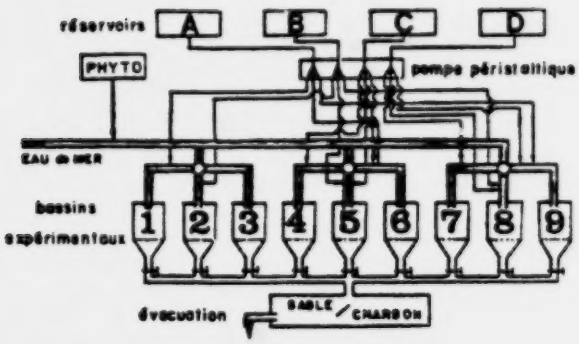

Fig. 1. Appareillage experimental utilisé pour réaliser la contamination des moules en laboratoire. A,B,C,D: Réservoirs de contaminants. 1-9: Bassins contenant les moules.

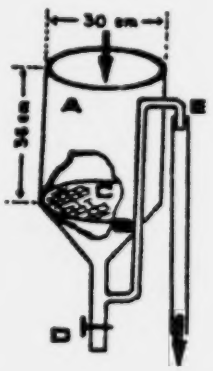

Fig. 2. A: Réservior. B: Entonnoir C: Grille de retenue pour les moules D: Boyau de vidange. E: Trop-plein.

Evaluation des activitiés de l'enzyme malique, de la glutamate déshydrogénase et de la glutathione peroxydase chez P. borealis.

L'homogénéisation du muscle abdominal de la crevette a été faite dans un milieu contenant 20 mM de triéthanolamine, 0.5 mM de PMSF, et 0.5 mM de dithiothréitol (DTT) à pH 7.6, avec un homogénéisateur Virtis pendant 2 min. en utilisant 2.5 mL de tampon par gramme de muscle abdominal. L'homogénat est laissé à reposer pendant 30 min. afin de permettre l'éclatement des mitochondries avant une centrifugation de 30 min à 24 000 g et 4°C. Le surnageant est conservé a -70°C jusqu'à son utilisation. L'enzyme malique a été utilisée dans le sens de la décarboxylation du malate (Lehninger 1982).

La glutamate déshydrogénase:

Cette enzyme présente peu d'activité dans le sens de la déshydrogénation du glutamate. Dans les conditions des présents essais, la réaction inverse, c'est-à-dire l'hydrogénation réductive du 2-cétoglutarate, est fortement favorisée. C'est donc elle qui à été étudiée en détail (Bergmeyer 1983). Les travaux de mise au point des niveaux d'activité de la glutathione peroxydase sont actuellement en cours.

Evaluation de la fragilité membranaire lysosomale:

La fragilité de la membrane lysosomale a été mesurée d'aprés la méthode décrite par Moore (1976). Cette méthode cytochimique se base sur le temps de préincubation à pH 4.5 nécessaire pour donner un maximum d'intensité de coloration pour l'enzyme N-acétyl-b-glucosaminidase. Cet indicateur de pollution a été utilisé pour étudier:

1. l'effet d'un déversement de Bunker C, sur la fragilité de la membrane lysosomale chez *M. edulis* echantillonnées sur le site du déversement (2 stations), à plusieurs mois d'intervalle, et dont les résultats ont été comparés à des moules prélevées sur un site contrôle à Pointe-Mitis;

2. l'effet de métaux lors de l'expérience réalisée en bassins et décrite ci - haut.

Methodes analytiques

Les tissus ont été digérés dans de l'acide nitrique concentré et le mercure total a été analysé en duplicata par absorption atomique sans flammes à l'aide d'un spectrophotomètre Perkin-Elmer model H6-3, selon la méthode déjà décrite par Pelletier (1986).

RESULTATS ET DISCUSSION

Tel qu'illustré aux Figs. 3 et 4, les niveaux de bioaccumulation du mercure diffèrent radicalement selon les doses utilisées. Les résultats d'expériences antérieures dans nos laboratoires ont démontré que les effets toxiques du mercure habituellement rencontres chez la moule bleue tels qu'une diminution de la vitesse de filtration, une diminution progressive de la production de féces (Watling and Watling 1982) et un taux élevé de mortalité aprés 30 jours de protocole expérimental, se développent en présence de doses supérieures à 30 µg/L de méthylmercure. Pour deux expériences réalisées, l'une à l'été 1986 (Fig. 3) et l'autre à l'été 1987 (Fig. 4), on peut observer que pour une dose sous-léthale de 3 µg/L, la bioaccumulation du mercure est direc-

tement proportionnelle à la concentration du polluant dans le milieu ambiant. Cette dose ne provoque pas la mort des organismes, ne modifie pas les poids des tissus mous, du manteau et du muscle adducteur postérieur, ni la concentration de protéines dans ces tissus. Il est intéressant de noter que pour des doses de 0.3 µg/L (Figs. 3 et 4) et de 0.01 µg/L (Fig. 4) les niveaux de bioaccumulation du mercure atteignent un plateau. Celui-ci est probablement le reflet de mécanismes de détoxification cellulaires comme l'induction de la synthèse de protéines liantes telles les métallo-protéines. Le destin d'un contaminant, même chez un organisme réputé pour être un bon indicateur de pollution, est donc différent selon le niveau de contamination.

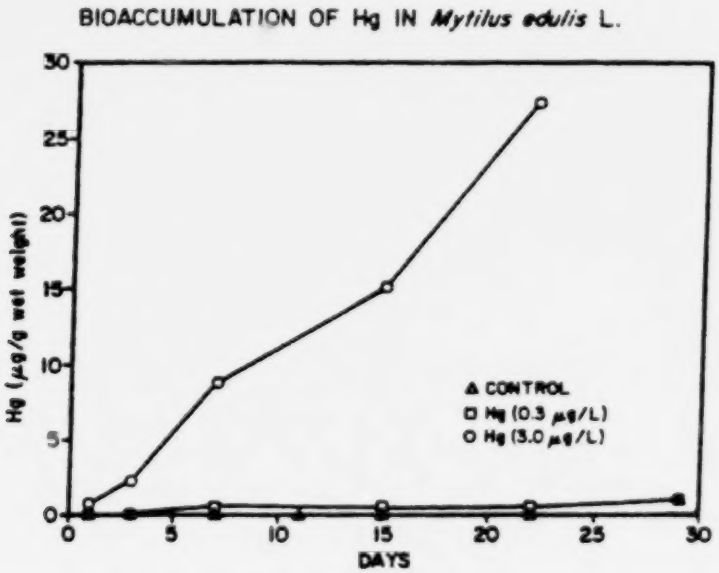

Fig. 3. Bioaccumulation de mercure total chez *M. edulis* mises en présence ou en absence de deux concentrations sous lèthales de méthylmercure pendant une période de 29 jours (été 1986).

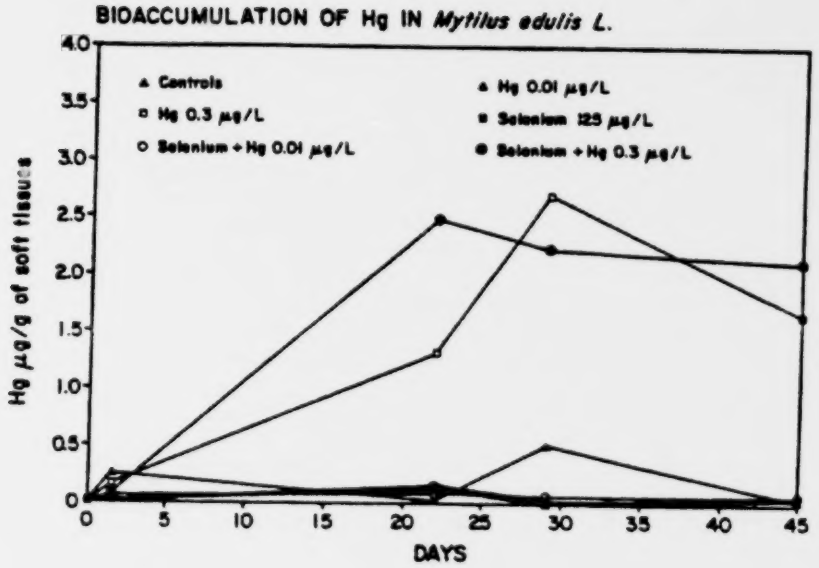

Fig. 4. Bioaccumulation du mercure total chez présence et en absence de sélénium pour une période de 45 jours (été 1987).

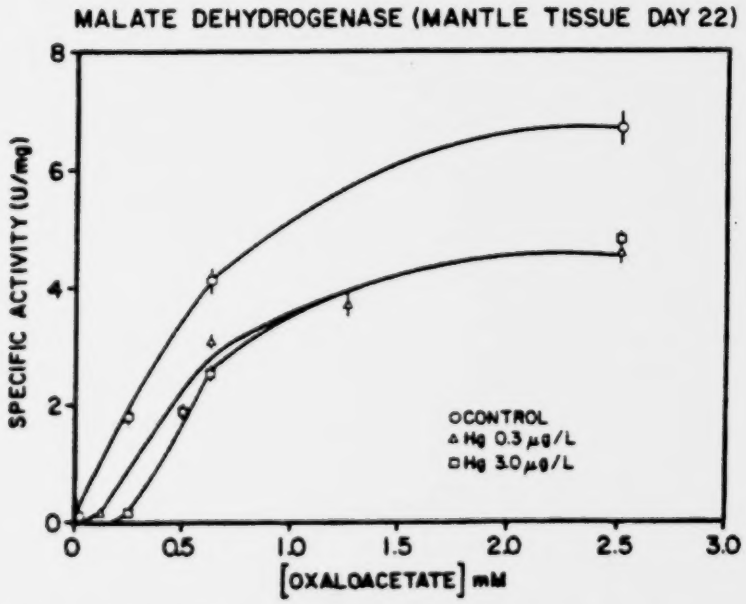

Fig. 5. Etude des effets de deux concentrations sous-léthales de méthylmercure sur l'activité spécifique de la malate déshydrogénase.

1985b; Wang et al. 1985b), part of the Hg released from the sediment may be attributed to the dissolution of the poorly crystalline oxides. In addition, the data on pH, Eh, and dissolved oxygen (Table 3) indicate that some other chemical and physical changes such as redox processes in the sediment systems could also have occurred. The Hg released from the sediments may be the combined effects of all the chemical and physical changes in the system. The desorption of Hg from the sediments appeared to be slightly faster at 25°C than at 4°C (Table 2). However, the effect of temperature on the Hg desorption rate was not substantial, if the experimental errors were taken into account.

The data in Table 4 show that the Hg released from the sediments decreased drastically when the pH of citric acid solution was not adjusted drastically before addition to the sediments regardless of the citric acid concentrations studies. The decrease of Hg from sediments with the decreasing pH was also observed by Jackson et al. (1980) and Zvonzrev and Zyrin (1983). Jackson et al. (1980) attributed the decrease in Hg released from the sediments at lower pH to the exceptionally strong covalent bonding between the Hg and humic matter, whereas Anderson (1967) indicated that the decrease in Hg release was due to the decreasing solubility of the humate-Hg complex at lower pH. The data obtained in the present study indicate that H^+ may interfere with the Hg release by retarding the complex formation between the sediment-bound Hg and citrate ligands and/or by coagulating the sediment suspensions which can significantly reduce the surface area and the amount of adsorbed Hg exposed to the citrate ligand. The coagulation of the sediment suspensions was visually observed immediately after the addition of citrate solutions with pH <4.1.

CONCLUSIONS

The Hg desorption from the lake sediments as influenced by citrate ligands obeyed the multiple first order kinetics. A fast desorption process occurred in the first hour, followed by a much slower desorption process. The rate of the Hg release from the sediments increased with increasing citrate ligand concentration especially at 10^{-3} M or higher. The increase in the rate and amount of Hg released from the sediments is attributed to the combined effects of complexation by Hg by citrate ligand, decreasing suspension Eh and dissolved oxygen, and increasing dissolution of the poorly crystalline oxides of Al, Fe, and Mn. The Hg desorption was negligible when the pH of the citric acid solutions were not preadjusted to 7.0 regardless of the citric acid concentrations studied. Temperature had little effect on the Hg released from the sediments by citrate ligand in the temperature range of 4 to 25°C.

ACKNOWLEDGEMENTS

This study was supported by the Natural Sciences and Engineering Research Council of Canada - Strategic Grant 32629 - Huang.

L'étude des variations d'activité de la MDH a été également faite lors d'un suivi d'élevage de moules à l'été 1986. Cet élevage présente à chaque année une période de mortalité importante au début d'août et nous avons voulu vérifier si notre indicateur biochimique pouvait détecter des épisodes de stress physiologiques au sein de cette population.

Comme on peut le voir sur la Fig. 7, l'activité de la MDH est diminueé de 50% dans le manteau de moules d'un an du 14/07/1986 au 25/08/1987 avec un déplacement du Km vers la droite de 7 fois démontrant ainsi un dérèglement de la liaison du substrat au site catalytique de l'enzyme. Les moules de deux ans pour leur part, ont une activité maximale de la MDH similaire pour les 5 dates d'échantillonnges mais avec un Km déplacé lui aussi de 7 fois vers la droite pour la période entre le 14 juillet et le 1 er août, avec une récupération évidente de l'activité catalytique enzymatique à la fin d'août 1986.

Ces résultats indiquent donc que:
- l'on peut caractériser l'activité enzymatique de la MDH selon l'âge de l'organisme étudié et selon le polluant étudié;
- que l'on peut détecter avant les périodes de stress intense, des variations métaboliques importantes au niveau de la MDH;
- que cet indicateur donne des réponses assez précises pour suivre le processus de récupération dans l'animal.

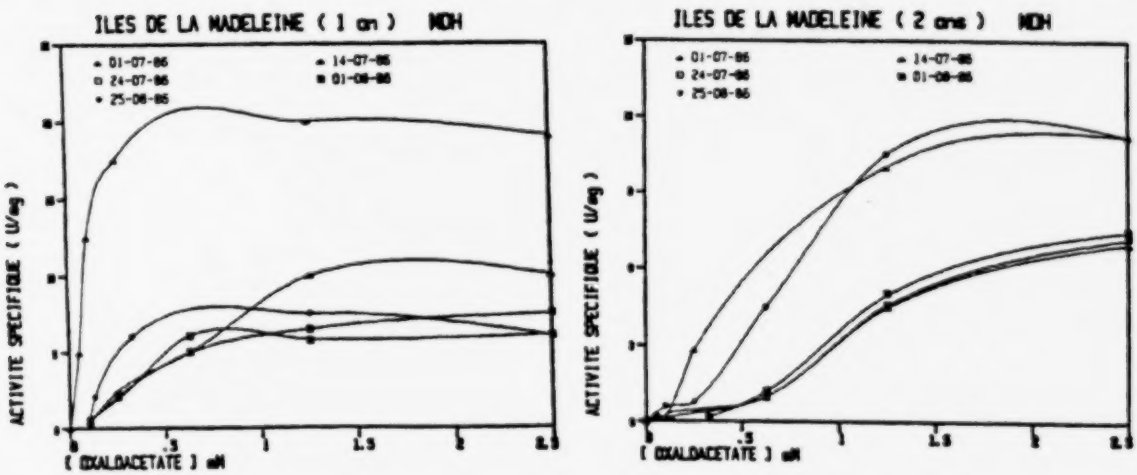

Fig. 7. Variations temporelles de l'activité spécifique de la malate déshydrogénase en fonction de concentrations croissants de substrat, au cours de l'été 1986.

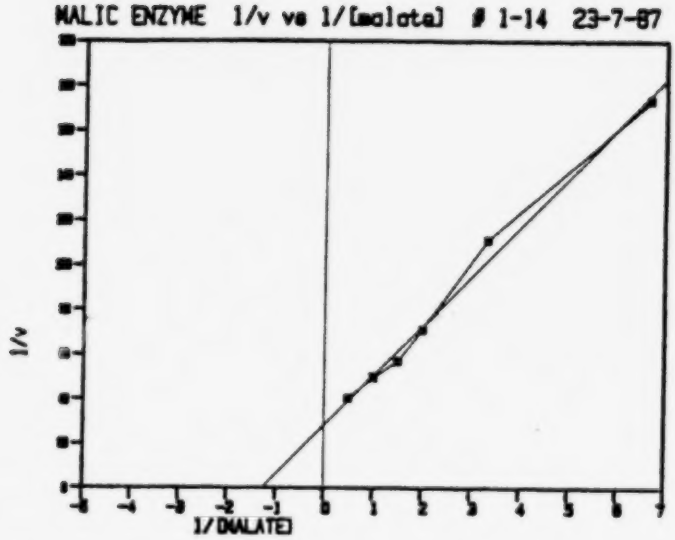

Fig. 8. Etude des parametres cinetiques de l'enzyme malique dans le muscle abdominal de la crevette nordique, *P. borealis*.

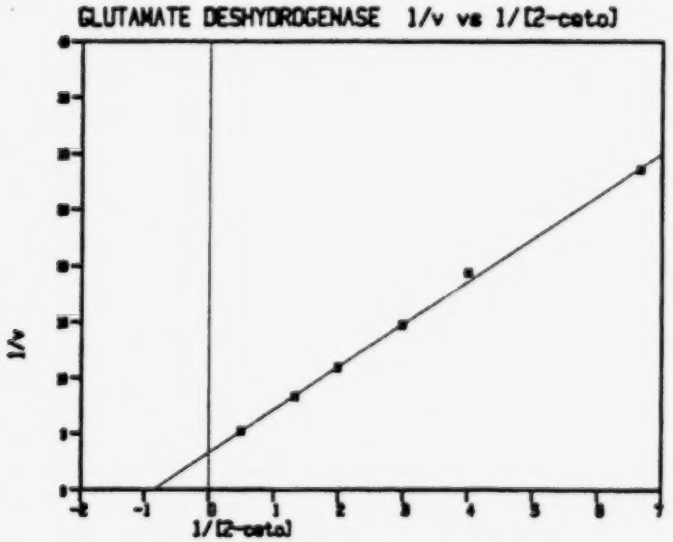

Fig. 9. Etude des parametres cinetiques de la glutamate déshydrogénase dans le muscle abdominal de la crevette nordique, *P. borealis*.

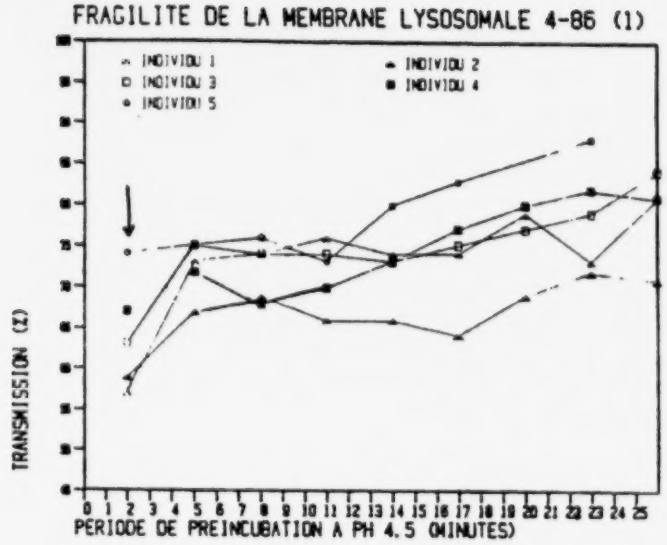

Fig. 10. Measure de la fragilité de la membrane lysosomale de l'hepatopancreas de moules échantillonnées en Avril 1986, au quai de Matane, a 1 km l'est du site d'echouage du Pointe Lévis et 4 mois aprés le deversement.

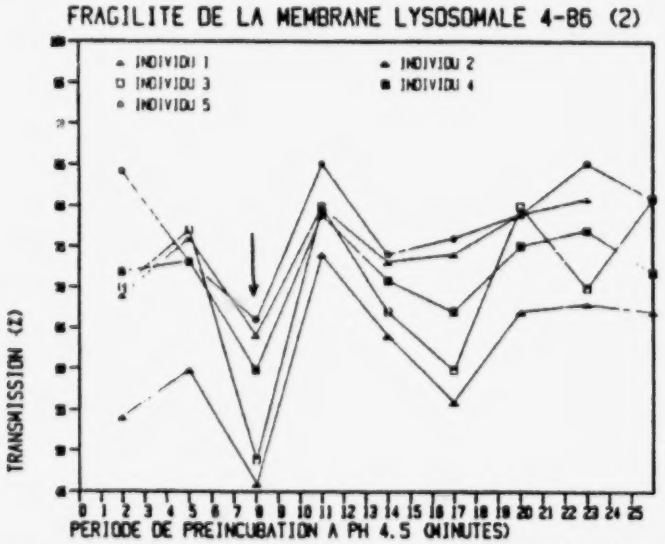

Fig. 11. Mesure de la fragilité de la membrane lysosomale de l'hepatopancreas de moules échantillonnées en Avril 1986, au site d'echouage du Pointe-Lévis, pres du phare de Mantane.

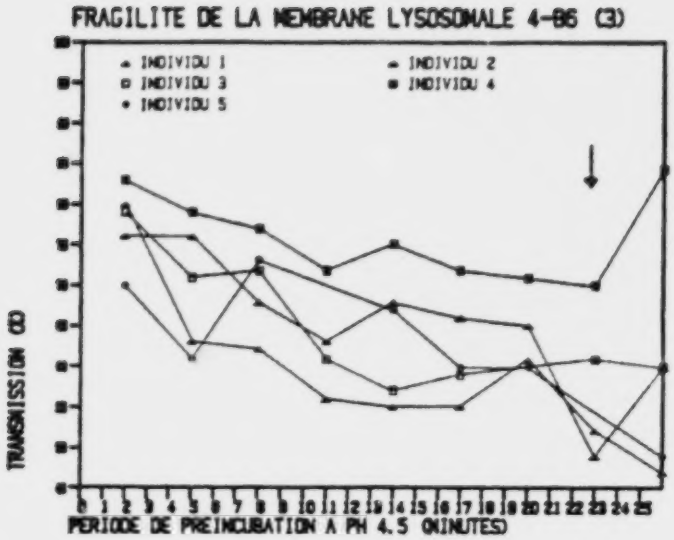

Fig. 12. Mesure de la fragilité de la membrane lysosomale chez moules échantillonneés dans un site contrôle a Pointe-Mitis, en Avril 1986.

Le développement d'indicateurs biochimiques de la qualité d'un environnement marin nécessite la mise en application de ces indicateurs chez plusieurs organismes. Nous sommes actuellement à mettre au point des indicateurs de doses sous-léthales de méthylmercure pour la crevette nordique *P. borealis*. Les études de cinétique enzymatique (Fig. 8) montrent que la réciproque de l'activité ne varie pas linéairement en fonction de la réciproque de la concentration en malate et ceci probablement à cause de la présence d'isoenzymes. La prochaine étape de mise au point sera d'isoler la fraction mitochondriale de l'homogénat du muscle abdominal de la crevette et de caractériser l'enzyme malique qui s'y trouve. La Fig. 9 illustre l'activité de la glutamate déshydrogénase (GDH) pour des conditions de pH 7.8, [triéthanolamine] 30 mM, [2 cétoglutarate] 2 mM, [CH_3 $COONH_4$] 150 mM, [ADP] 0.75 mM. Sous ces conditions l'activité spécifique de la GDH du muscle abdominal de la crevette provenant du Saguenay est de 0.207±0.0035 uM de NADH par mg de protéines. La mise au point et la caractérisation de la glutathione peroxydase chez *P. borealis* est actuellement en cours. Nous prévoyons utiliser ces trois enzymes lors d'expériences avec des crevettes du Golfe St-Laurent d'une part et avec des crevettes du Saguenay d'autre part, en vue de comparer les activités enzymatiques en fonction d'une contamination chronique au mercure. Chaque expérience se fera sur trois groupes de crevettes nourries avec des moules contaminées au méthymercure, au sélénium et aux deux polluants pour le dernier groupe. Ceci devrait nous permettre d'établir l'utilité de chacun des enzymes comme indicateurs biochimiques chez la crevette, d'établir s'il y na un antagonisme sélénium-mercure chez la crevette et d'observer le processus d'acclimatation au niveau métabolique de crevettes contaminées de facon chronique dans la rivière Saguenay et ceci en comparant avec des crevettes temins du Golfe St-Laurent. Pour compléter la mise au point d'indicateurs de pollution sous-léthale, nous avons fait la mesure de la fragilité de la membrane lysosomale chez la moule bleue.

Nous avons utilisé cet indicateur de pollution dans deux expériences différentes. Dans la première, nous voulions étudier l'effet du déversement de Bunker C lors de l'échouage du Pointe-Lévis au début du mois de décembre 1985, sur la fragilité de la membrane lysosomale chez des organismes (*M. edulis*), en milieu naturel. Les moules ont été prélevées à deux endroits differents prés du site de déversement, à plusieurs mois d'intervalle et à un site contrôle à Pointe-Mitis. Des exemples des résultats obtenus sont illustrés dans les Figs. 10, 11 et 12 et ces résultats correspondent à l'échantillonnage du mois d'avril 1986, les glaces présentes nous ayant empêché d'échantillonner entre décembre et avril. La Fig. 10 illustre la réponse de la membrane lysosomale au site 1 d'échantillonnage situé sur la côte prés du phare de Matane, vis-à-vis le site du déversement de Bunker C. La réponse des membranes lysosomales à ce site indique un état important de stress démontré par la grande perméabilité de la membrane qu présente un maximum de coloration aprés 2 minutes d'incubation. A 1 km du site de déversement, près du quai de Matane, le pétrole ayant été amené grâce aux forts vents d'ouest lors de cette tempête, les moules présentent un état de stress un peu moins prononcé (Fig. 11; 8 minutes) qu'au niveau du quai mais beaucoup plus important qu'à la station contrôle (Fig. 12) où le temps de préincubation oscille entre 23 et 26 minutes. Ces dernières valeurs sont consideree comme reflétant un état physiologique normal. Cet indicateur général d'une condition de stress chez la moule mise en présence d'hydrocarbures, s'est avéré également utile pour etablir les conditions de stress des moules mises en bassin et contamiées au mercure et au sélénium. Des résultats préliminaires indiquent que le stress occasionné par la mise des moules en bassins au laboratoire, ainsi que de la présence de métaux et de la récupération des moules au stress peuvent être detectespar cet indicateur cytochimique.

CONCLUSION

Les indicateurs actuels de toxicité doivent être complétés par des indicateurs plus sensibles aux concentrations sous-léthales des polluants. Les indicateurs biochimiques tels l'étude des paramètres cinétiques d'enzymes-clés du métabolisme des organismes choisis comme bioindicateurs ainsi que l'élaboration d'un indicateur général de stress environnemental, par la mesure de la fragilité de la membrane lysosomale, nous semblent un choix réaliste pour suivre et évaluer la qualité d'un environnement marin.

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THE DEVELOPMENT OF A PROTEIN SYNTHESIS ASSAY FOR MONITORING THE ENVIRONMENTAL HEALTH OF FISH EXPOSED TO HEAVY METALS SUCH AS MERCURY

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ABSTRACT

A key to managing and preventing contaminant effects on fish populations is a capacity for early warning. If an important population response arises from a sequence of molecular responses, tests of functions that respond rapidly to contaminant exposure will be the most useful as diagnostic tools. In addition, this response must persist, otherwise physiological compensation or homeostasis will decrease the likelihood of detecting contaminant effects.

A cell-free protein synthesis assay was developed to study the chronic toxic response of rainbow trout to low levels of inorganic Hg. Laboratory studies were undertaken to evaluate the biological sensitivity of this test system, including the development of dose-response relationships based on ambient water concentrations and tissue contaminant levels.

The translational activities of *liver* ribosomes were significantly higher ($p<0.05$) in Hg treated fish exposed to 5.0, 2.0 and 0.2 μg Hg^{2+}/L for 14 d then control animals. Exposure of fish to 5.0 μg Hg^{2+}/L produced a muscle residue level of approximately 0.2 mg/kg and a 30 percent increase in liver protein synthesis.

The incorporation of radiolabelled amino acid into peptide was significantly higher in the liver ribosomes of fish exposed to 5.0, 50.0 and 100.0 μg Hg^{2+}/L for 4 d, and in fish exposed to 0.04 or 0.1 μg Hg^{2+}/L for 24 h relative to control fish. These results suggest the usefulness of this assay for monitoring the rapid response of fish to low levels of Hg. This property may be particularly useful for monitoring rapid environmental fluxes of Hg pollution in the field.

In conclusion, the response of rainbow trout to low levels of Hg can be effectively measured by a cell-free protein synthesis assay. Investigating the translational activity of liver ribosomes provides a rapid and sensitive biochemical response. Further studies are being undertaken to investigate the synthesis of metallothionein and other putative stress-induced proteins. Toxicological evidence indicates that there are important biological costs associated with increased production of these proteins. [This research was supported by an Ontario Ministry of the Environment grant.]

RÉSUMÉ

La capacité d'une détection hâtive constitue la clé de la prévention et de la gestion des effets exercés par des contaminants sur des populations de poissons. Lorsqu'une population réagit considérablement à une séquence de réactions moléculaires, alors l'examen de fonctions qui manifest nt très tôt une réaction à l'exposition à des contaminants constituera l'instrument de diagnostic le plus utile. En outre, cette réaction doit persister, faute de quoi des mécanismes de compensation physiologique

ou l'homéostasie vont abaisser la probabilité de détection des effets exercés par les contaminants.

Un test de synthèse protéinique sans substrat cellulaire a été mis au point pour étudier la réaction de truites arc-en-ciel à une intoxication chronique au mercure inorganique à faible concentration. Des expériences en laboratoire ont été entreprises afin d'évaluer la sensibilité biologique de ce système, notamment le développement des rapports dose-effet fondé sur les concentrations de contaminants dans l'eau ambiante et dans les tissues.

L'activité des ribosomes du *foie* pour la translocation était significativement plus élevée ($p<0.05$) chez les poissons traités au Hg et exposés à 5.0, 2.0 et 0.2 Hg^{2+}/L pendant 14 jours que chez les témoins. L'exposition des poissons à 5.0 μg Hg^{2+}/L a conduit à niveau résiduel dans les muscles d'environ 0.2 ppm et à une augmentation de 30% de la synthèse protéinique dan le foie.

L'incorporation dans des peptides d'acides aminés marqués avec un produit radioactif était significantivement plus élevée dans les ribosomes du foie de poissons exposés à 5.0, 50.0 et 100.0 μg Hg^{2+}/L pendant 4 jours ainsi que chez des poissons exposés à 0.04 ou 0.1 μg Hg^{2+}/L pendant 24 h, qu'elle ne l'était témoins.

Les résultats montrent l'efficacité de ce test se détection de la réaction hâtive de poissons à de faibles concentrations de Hg. Cette propriété peut se révéler particuliérement utile pour la surveillance sur le terrain des mouvements rapides de pollution au Hg.

En conclusion, la réaction de truites arc-en-ciel à de faibles concentrations de mercure peut être mesurée de façon efficace par un test de synthèse protéinique sans substrat cellulaire. L'examen de l'activité des ribosomes du foie pour la translocation permet d'évaluer une réaction biochimique rapide et sensible. D'autres études sont en cours pour examiner la synthèse de la métallothionéine et d'autres protéines qu'on suppose induites par un stress. Les résultats de recherches toxicologiques indiquent qu'il y a des coûts biologiques importants associés à une production accure de ces protéines. (Une subvention du ministère de l'Environnement de l'Ontario a été accordée pour cette recherche.)

INTRODUCTION

Surveillance of aquatic biota is required for assessing the environmental impact of trace contaminants. In addition to measurements of tissue contaminants levels, measurements of chronic biochemical responses are desirable to assess the toxicological importance of contaminant residues. Knowledge of the mechanisms with which animals handle toxic substances is necessary if we are to understand the response and tolerance of the biota to these substances.

A key to managing and preventing contaminant effects on fish populations is a capacity for early warning (NRCC 1985). If an important population response arises from a sequence of molecular responses, tests of functions that respond rapidly to contaminant exposure will be the most useful as diagnostic tools. In addition, this response must persist, otherwise physiological compensation or homeostasis will decrease the likelihood of detecting contaminant effects. Clinical tests to determine the general state of health, to evaluate the physiological effects of toxicants and

drugs, and to diagnose specific diseases are well established in human and veterinary medicine. However, they have become established only through extensive research correlating physiological and biochemical responses with whole-animal responses.

Mercury affects mammalian protein synthesis in various target organs (Das et al. 1982; Berlin 1986). At relatively low doses, mercury stimulates protein synthesis and can stimulate the production of various classes of proteins such as metallothioneins and the acute phase reactants (Samji et al. 1985; Harrison and Nicholls 1986). At high doses, or small doses over longer periods, structural disorganization of the protein synthetic machinery or ribosomal loss has been correlated with the inability of many tissues in mercury poisoned animals to incorporate amino acids into proteins (Cavanagh 1983).

This report discusses the usefulness and the toxicological importance of a cell-free protein synthesis assay for monitoring the chronic response of rainbow trout exposed to inorganic mercury. This liver assay probably represents the first time this experimental approach has been utilized in the field of aquatic toxicology. In order to evaluate the biological sensitivity of this test system, laboratory experiments were designed to investigate dose-response relationships and the response time and its duration.

MATERIALS AND METHODS

Juvenile rainbow trout (*Salmo gairdneri*), total length of approximately 110-270 mm, were exposed to different ambient mercuric chloride concentrations for various lengths of time. The fish were fed daily during these continuous flow tests. The fish were tested in 20 L plastic buckets, using 15°C de-chlorinated Toronto tap water as dilution water, in accordance with the specifications outlined by Sprague (1973). All fish exposure conditions did not vary by more than 10%. In general, each bucket or sample contained one fish unless otherwise indicated.

The standard ribosomal assay, as described by Kuliszewski and Nicholls (1983), was employed. Excess amounts of control liver postmicrosomal supernatant fraction (source of elongation factors) were incubated with limiting amounts of ribosomes (25-100 μg RNA/tube) bearing endogenous mRNA, from control and mercury treated fish.

The incorporation of radiolabelled leucine into peptide was carried out for 30 min at 25°C. The reaction was stopped and the proteins were precipitated, washed and counted in a liquid scintillation counter.

Water and fish mercury concentrations were determined by flameless atomic absorption spectrophotometry (OME, 1981). RNA concentrations were determined by taking a ferritin correction factor into account (Campbell and Sargent 1969) and using absorbency:

$$A_{260nm}^{1\ cm}\ 1.00 = 50\ \mu g/ml.$$

Statistically significant differences between two means were determined by t-tests (Sokal and Rohlf 1981). Probability (P) values of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The polysomal fractions prepared from the livers of control and mercury treated fish were tested in a protein synthesizing system. At all levels of polysomes tested, fish exposed to 5 μg Hg^{2}_{+}/L for 14 d exhibited a large and statistically significant increase in the incorporation of [^{14}C] leucine into peptide (Fig. 1). This produced a 30% increase in liver protein synthesis. The concentration of mercury in the livers of the mercury treated and the control fish were approximately 2,000 and 40 ng Hg/g, respectively.

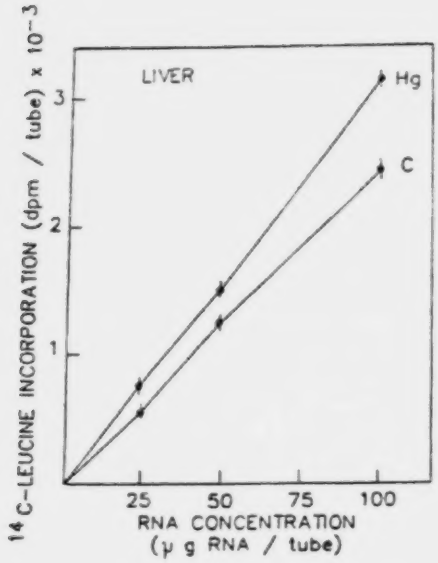

Fig. 1. Synthesis of protein, as monitored by the incorporation of [^{14}C] leucine into peptide by liver ribosomes isolated from control and mercury treated fish exposed to 5.0 μg Hg^{2+}/L for 14 d. Each value represents the mean±SE of the mean of 3 separate experiments (n=7).

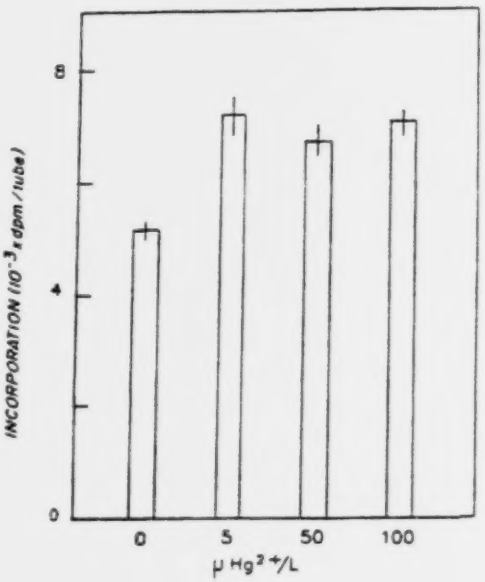

Fig. 2. The incorporation of [^{14}C] leucine into peptide in a cell-free assay, using liver ribosomes (100 μg RNA/tube) isolate from control and mercury treated fish exposed to varying levels of Hg^{2+} for 4 d. Mean±SEM for 19, 6, 5, and 6 experiments, respectively, for exposures of 0, 5, 50 and 100 μg Hg^{2+}/L.

The translational activities of liver ribosomes were significantly higher in fish exposed to 2 and 0.2 μg Hg^{2+}/L for 14 d than in control animals (Tables 1 and 2). Similarly, fish exposed to 5, 50 and 100 μg Hg^{2+}/L for four days exhibited a significantly higher incorporation of radiolabelled leucine into peptide than control animals (Fig. 2).

Table 1. Incorporation of [^{14}C] leucine into peptide *in vitro* using control liver supernatant fraction and ribosomes (100 μg RNA/tube) from control and mercury treated fish (2.0 μg Hg^{2+}/L) after 14 d of exposure. Each value represents the mean and the standard deviation of 4 replicate samples. Each sample consisted of 2 pooled livers.

Hg concentration (μg Hg^{2+}/L)	Polysomes (μg RNA/tube)	Incorporation (DPM/tube x 10^{-3})
0.0	100	5.528±0.163
2.0	100	*7.213±0.968

* $p<0.05$

The toxicological importance of these results is based on the knowledge that these levels of inorganic mercury produced muscle residue levels comparable to those found in Ontario sports fish and the induction of liver protein synthesis may be a useful early warning indicator of fish toxicity (Table 3). In addition, the proposed maximum allowable concentration in American drinking water supplies is 3.0 μg/L of mercury (Thompson 1986).

Table 2. Incorporation of [^{14}C] leucine into peptide *in vitro* using control liver supernatant fraction and ribosomes (100 μg RNA/tube) from control and mercury treated fish (0.2 μg Hg^{2+}/L) after 14 d of exposure. Each value represents the mean and standard deviation of 3 replicate samples. Each sample consisted of 4 pooled juvenile fish livers.

Hg concentration (μg Hg^{2+}/L)	Incorporation (DPM/tube x 10^{-3})
0.0	6.750±0.393
0.2	*10.194±1.585

* $p<0.05$

Table 3. Mercury levels (means±SD) in fish muscle.

Exposure period (d)	Hg water conc. (μg Hg^{2+}/L)	Hg muscle conc. (ng Hg/g)	(n)
	Control	29±13	(8)
4	5	105±21	(4)
14	5	220±20	(3)
4	50	1575±562	(4)
4	100	1825±562	(4)

The exposure of fish to 5 μg Hg^{2+}/L for 14 d produced a muscle residue level of 220±20 ng Hg/g. Similar mercury concentrations in yearling yellow perch have been shown to correlate with reduced growth of perch in Ontario lakes (Suns et al. 1987). Laboratory studies have also reported reduced growth of fish exposed to inorganic mercury levels of 5 μg/L of less (U.S. EPA 1983; Snarski and Olson 1982; Brown and Parson 1978; Christensen 1975).

It is possible that the induction of liver protein synthesis plays an important role in affecting fish growth. The biochemical response exhibited by rainbow trout was shown to persist for at least 14 d. Although more research is required to resolve this issue, there is some evidence that indicates that increased production of liver metallo-proteins and the development of a tolerance response is associated with reduced growth rates of fish (Buckley et al. 1982; Chapman 1985; Dixon and Sprague 1981). The two events may have a partial cause-effect relationship because of the metabolic demands of the acclimation process (Chapman 1985).

Proposed biological functions for metallo-proteins include the storage and transport of essential metals (e.g., Cu and Zn), and the detoxification of these and the non-essential metals, Hg and Cd (NRCC 1985). Sublethal concentrations of these metals generally produce elevated liver metallothionein concentrations and acclimation to heavy metal toxicity within a wk or less.

Young fish exposed to 0.04 and 0.1 μg Hg^{2+}/L for 24 h also exhibited a significantly higher incorporation of radiolabelled leucine into peptide relative to control fish (Table 4). These mercury concentrations are below the U.S. EPA (1983) water quality criteria for the protection of aquatic life and these results indicate the usefulness of this assay for monitoring the rapid response of fish exposed to low levels of mercury. This property may be particularly useful for monitoring rapid environmental fluxes of mercury pollution in the field.

The experimental approach employed in this study is amenable to investigating net changes in liver protein synthesis. Rainbow trout exposed to 5, 50 and 100 μg Hg^{2+}/L for 96 h did not exhibit significant differences in peptide synthesis (Fig. 2). These results suggest that the synthesis of metal-induced proteins remains constant over the range of mercury concentrations tested.

Table 4. Incorporation of [^{14}C] leucine into peptide *in vitro* using control liver supernatant fraction and ribosomes (25 or 100 μg RNA/tube) from control and mercury treated fish (0.04 and 0.10 μg Hg^{2+}/L) after 24 h of exposure. The number of replicate samples are shown in parenthesis. Each sample consisted of 5 pooled juvenile fish livers (mean±SD).

Hg concentration (μg Hg^{2+}/L)	Polysomes (μg RNA/tube)	Incorporation (DPM/tube x 10^{-3})	(n)
0.00	25	1.779±0.205	(4)
0.00	100	4.902±0.352	(3)
0.04	25	*2.917±0.637	(3)
0.04	100	*6.923±1.132	(3)
0.10	25	*2.852±0.209	(2)
0.10	100	5.701±0.44	(2)

Future studies are required to investigate the products of translation and to further elucidate the stress response exhibited by rainbow trout. Preliminary results of electrophoretic protein analysis show an increase in the relative labelling of a 10-15,000 dalton polypeptide in fish exposed to 50 μg Hg^{2+}/L for one wk. This could possibly represent metallothionein synthesis. Any dose-dependant changes in metallo-protein synthesis during heavy metal toxicity is of major interest to aquatic toxicologists.

In conclusion, the response of rainbow trout exposed to low levels of mercury can be effectively measured by a cell-free protein synthesis assay. Investigating the translational activity of liver ribosomes, provides a rapid and sensitive biochemical response. Future studies involving the testing of natural fish populations would be useful to extend these findings.

ACKNOWLEDGEMENT

This research was supported by an Ontario Ministry of the Environment grant.

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LE MECHANISME BIOCHIMIQUE DE RECUPERATION EN *SELENASTRUM CAPRICORNUTUM* EXPOSE A CADIUM: LE METABOLISME ADENYLATE ET LA SYNTHESE DES MACROMOLECULES

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RÉSUMÉ

La recherche sur les effets exercés par les métaux lourds sur la photophosphorylation et al phosphorylation oxydative phytoplanctonique a contributé à l'élucidation des mécanismes responsables de formes d'inhibition de la croissance observées quand des métaux toxiques sont présents. Le rôle de ces deux voies métaboliques dans le processus de récupération (acclimation) qui suit l'exposition à un toxique n'a pas encore été élucidé. La présente étude vise à l'examen de la relation entre le métabolisme en tremes énergétiques et le taux de croissance durant la période de récupération.

Des cultures par lots de *Selenastrum capricornutum* dans un milieu AAP modifié à pH 7.2 ont été exposées à 30 et 100 µg Cd/L. Après une exposition de 48 h au Cd, 2.67 x 10^{-6} M EDTA a été ajouté afin de complexer 97% de l'ion Cd, ce qui réduisait par le fait même la toxicité initiale du métal. Les résultats montrent que la toxicité est liée au taux d'assimilation du Cd. Des variations dans les quotients molaires d'adénylate (charge énergétique, ATP/AMP, ATP/ADP) et dans le taux d'incorporation du carbone dans les macromolécules, sont en rapport avec le taux de croissance et peuvent constituer des indicateurs de prévision de la récupération.

ABSTRACT

Research on the effects of heavy metals on phytoplankton photo-phosphorylation and oxidative phosphorylation has contributed to the understanding of the mechanisms responsible for the growth inhibitions observed in the presence of toxic metals. The role of these two metabolic pathways in the process of recovery (acclimation) following exposure to a toxicant is still not clear. The aim of the present study is to investigate the relationship between energy metabolism, and growth rate during recovery.

Selenastrum capricornutum batch cultures grown in modified AAP medium at pH 7.2 were exposed to 30 and 100 ug/L Cd. After 48 h exposure to Cd, 2.67 x 10^{-6} M EDTA was added to complex 97% of the Cd ion, thereby reducing the initial toxicity of the metal. Results show that toxicity is linked to the rate of Cd uptake. Variations in the adenylate molar ratios (energy charge, ATP/AMP, ATP/ADP) and the

rate of carbon incorporation into macromolecules are related to growth rate and can be used as indicators to predict recovery.

ALTERATIONS IN SERUM CHEMISTRY IN RAINBOW TROUT (*SALMO GAIRDNERI*) WITH LIVER DEGENERATION AFTER PARTIAL HEPATECTOMY OR TREATMENT WITH CARBON TETRACHLORIDE OR ALPHA-NAPHTHYLISOTHIOCYANATE

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ABSTRACT

A variety of serum chemistry parameters were investigated to determine if biliary disease could be differentiated from hepatocellular disease in fish. Alpha- naphthylisothiocyanate (ANIT), at 200 mg/kg IP, induced bile duct and kidney epithelial necrosis, whereas CCl_4 and partial hepatectomy produce diffuse or local necrosis of liver parenchyma respectively. Many changes in serum electrolytes, proteins, and enzymes were observed but evaluation in alkaline phosphatase levels was most characteristic of biliary disease induced by ANIT. Hepatic necrosis by CCl_4 increased many of the same parameters as ANIT, including alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase, but notably alkaline phosphatase activities were decreased. Partial hepatectomy increased all these enzymes except alkaline phosphatase. The increase in serum alkaline phosphatase but not bilirubin or gamma-glutamyltranspeptidase in trout are different from changes observed in mammals with ANIT induced biliary disease.

RÉSUMÉ

Plusieurs paramètres de la chimie sérique ont fait l'objet d'examens visant à déterminer si la bilharziose pouvait être distinguée de la maladie hépatocellulaire chez le poisson. L'alpha-naphthylisothiocyanate (200 mg/kg IP) induit la nécrose épithéliale du foie et du canal cholédoque alors que le CCl_4 et l'excision partielle du foie produisent une nécrose diffuse ou locale du parenchyme du doie, respectivement. Beaucoup be changements sériques ont été observés pour les électrolytes, les protéines et les enzymes, mais l'évaluation faite de la concentration en phosphatase alcaline était tout à fait caractéristique de la bilharziose induite par l'ANIT. La nécrose du foie par le CCl_4 a fait augmenter beaucoup des mêmes paramètres que l'ANIT, notamment l'alanine - aminotransférase, l'aspartate - aminotransférase et la lactate - déshydrogénase, mais il faut insister sur l'activité réduite de la phosphatase alcaline. L'excision partielle du foie a accru la concentration de toutes ces enzymes à l'exception de la phosphatase alcaline. L'augmentation de la phosphatase alcaline sérique, mais pas de la bilirubine ou de la gamma - glutamyltranspeptidase chez la truite constitue une réponse différente de ce qui est observé chez les mammifères chez on a induit la bilharziose à ANIT.

MEDAKA SENSITIVITY TO TRICHLOROETHYLENE AS AFFECTED BY SIZE

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ABSTRACT

In preliminary experiments Japanese medaka (*Oryzias latipes*) of varying age were exposed under conditions of intermittent flow to trichloroethylene (TCE) for 7 d and their responses evaluated utilizing lethality as an end point. Fish averaging 11.0 mg blotted wet weight reflected a 7-d LC50 of 26.8 mg/L, while those averaging 4.0 mg produced a LC50 of 37.7 mg/L. The highest TCE concentration of 37.9 mg/L was insufficient to produce a calculable LC50 for the smallest fry used (1.3 mg). More thorough evaluations currently in progress support the preliminary indications that medaka sensitivity to TCE increases in direct proportion to fry weight. These studies involve medaka ranging in age from 4 d to one yr and indicate that sensitivity to TCE essentially doubles from an LC50 of 44 mg/L to one of 25 mg/L as the fish increase in size from 2 to 15 mg and that further growth does not result in an additional increase in sensitivity. [This study was supported in part by Public Health Service Contract NO1-CP-61070 from the National Cancer Institute and the U.S. Army Medical Research and Development Command.]

RÉSUMÉ

Lors d'expériences préliminaires, des "Japanese medaka" *Oryzias latipes* d'âge variable ont été exposés par circulation intermittente au trichloroéthyléne (TCE) pendant 7 jours; les réactions ont été évaluées en utilisant la léthalité pour terminer l'expérience. Des poissons ayant en moyenne 11.0 mg de poids frais "blotted" indiquaient une CL50 7 jours de 26.8 mg/L, tandis que ceux dont le poids moyen atteignait 4.0 mg avaient une CL50 de 37.7 mg/L. La plus forte concentration de TCE, 37.9 mg/L, n'a pas suffi pour produire une CL50 calculable chez le plus petit fretin utilisé pour les tests (1.3 mg). Des évaluations détaillées sont en cours et confirment les résultats préliminaires, soit qie la sensibilité d'*Oryzias latipes* au TCE augmente en proportion directe avec le poids du fretin. Ces études ont été faites sur des sujets vieux de 4 jours à 1 an et elles indiquent que la sensibilité au TCE double essentiellement, d'nue CL50 de 44 mg/L à 25 mg/L quand le poisson passe de 2 à 15 mg; en outre, toute croissance ultérieure ne se traduit pas par une augmentation additionelle de sensibilité. (Cette étude a été rendue possible en partie par le contact de service pour la santé publique NO1-CP-61070 du *National Cancer Institute* et par l'*U.S. Army Medical Research and Development Command*.)

HAZARDOUS SUBSTANCES OBJECTIVES FOR PROTECTION OF THE QUALITY OF SURFACE WATERS

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ABSTRACT

Owing to their toxic properties, many substances contained in municipal sewage and industrial waste water cause undesired changes in the composition of aquatic biocoenoses. This leads to a conflict of interests as to the use of these waters for certain purposes so that given objectives to protect the catchment of drinking and industrial water, fish farming and hatching, leisure and recreation areas, the marine environment as well as the aquatic biocoenoses can no longer be achieved.

The objectives as to the quality of water, in particular with respect to "hazardous substances" are essentially based on sublethal bioassays and so-called effect data (e.g. NOEC values). An evaluation of the chemical and physical data measured can only be made when these effect data obtained with the biotest methods are used which have already been largely standardized and intercalibrated. The determination of these effect data is necessary for risk assessment and consequently serves to protect the basic functions such as metabolic pathways, growth, movement, reproduction and genetic alterations as well as regulatory performances of aquatic biocoenoses concerning function and structure.

Preventative care, i.e. the determination and evaluation of a threshold value which should not be exceeded in order to avoid possible damage, is of primary importance. If the emission limits are insufficient to minimize the polluted input after clean-up strategies (using the best technical means), further requirements are to be fulfilled in accordance with the emission principle. These emissions are the hazardous substances which have not been removed by the best technical means and remain in the waterways. Here is a need for additional regulations based on sublethal bioassays. The effect-related biotest methods make an important contribution to express the requirements for corresponding regulations. By the introduction of certain parameters such as acute and chronic toxicity values to evaluate the risk of hazardous substances in the aquatic environment, a quantification according to the "principle of concern" is possible and should be followed by political decisions.

RÉSUMÉ

Á cause de leurs propriétés toxiques, beaucoup de substances contenues dans les eaux d'égout municipales et dans les effluents industriels provoquent des changements qui ne sont pas souhaités dans la composition des biocénoses aquatiques. Cela conduit à un conflit d'intérêts quant à l'utilisation de ces eaux à certaines fins; c'est ainsi que des objectifs donnés de protection des points d'approvisionnement en eau potable et en eau industrielle, et de protection de l'eau de pisciculture, des secteurs récréatifs, du milieu marin ainsi ques des biocénoses aquatiques, ne sont plus possibles.

Les objectifs de qualité de l'eau, notamment pour ce qui est des "matiéres dangereuses" sont fondés essentiellement sur des essais biologiques au niveau sublétal et sur ce qu'on a appelé les données sur les effets (ρ. ex., concentration sans effet ob-

servé). Une évaluation des données physiques et chimiques ne peut être faite que sur des résultats obtenus au bout d'essais biologiques dans une forme très normalisée et après intercalibration. Il est nécessaire d'obtenir ces données sur les effets pour l'evaluation des risques; éventuellement, cela sert à la protection des grandes fonctions comme les voies métaboliques, la croissance, le mouvement, la reproduction et du code génétique ainsi qu'a la protection des régulateurs des biocénoses aquatiques, en termes de fonction et de structure.

La prévention, c'est-à-dire la détermination et l'évaluation d'un plafond à ne pas dépasser si l'on veut éviter des dommages possibles, est de la plus grande importance. Á supposer que les limites fixées pour l'enlévement ne suffisent pas à minimiser l'apport de pollution après l'application des stratégies de nettoyage (avec les meilleurs moyens techniques possibles), alors il faut passes à d'autres exigences conformément au principe des "immissions". Ces "immissions" sont les substances dangereuses qui n'ont pas été extraites avec les meilleurs moyens techniques et qui resent présentes dans les voies d'eau. Il faut donc d'autres règlements dont les normes seront fondées sur des essais biologiques qui se rapportent aux effets sublétaux. Ce genre d'essais aide beaucoup à préciser les exigences arrîtées dans ces règlements. Avec l'introduction de certains paramètres comme les niveaux de toxicité aigues et chronique pour l'évaluation du risque lié à des substances dangeureuses dans les milieux aquatiques. il est possible d'obtenir des résultats quantifiés selon le 'principle du degré d'inquiétude"; ces derniers appellemt des décisions politiques.

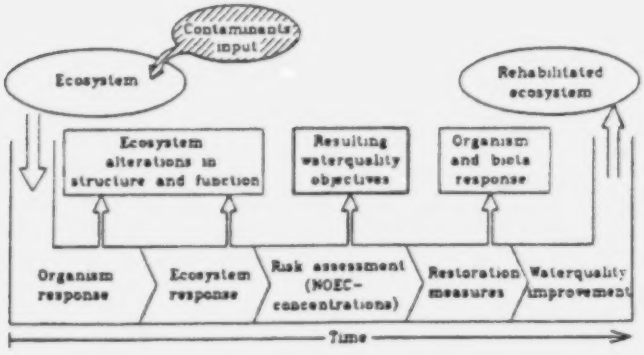

Fig. 1. Schematic representation of the input of anthropogenic substances from municipal sewage and industrial waste water in lakes and rivers: Risk assessment using sublethal effect-related bioassays (NOEC values = No Observed Effect-Concentrations) and time-dependent measures for rehabilitation of lakes or rivers.

A MODEL OF ORGANIC CHEMICAL BIOACCUMULATION BY FISH

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ABSTRACT

A comprehensive model available on a microcomputer is described which is based on the fugacity modelling concept and which contains expressions for the following processes or effects:

Chemical bioavailability in water as influenced by sorption,
Uptake and clearance by gill transfer,
Food uptake, digestion, and excretion,
Metabolism,
Fish growth.

The model parameters are discussed and it is demonstrated that biomagnification may occur with successive concentration increases as the food chain is ascended. A "fall off" in bioaccumulation is predicted for very hydrophobic chemicals.

The model yields either an analytical expression (equation) for concentration as a function of time, or, for more complex systems, it can be integrated numerically to give a tubular or graphical data. It is regarded as being particularly convenient for "fitting" exponential experimental data for a series of compounds of varying hydrophobicity.

RÉSUMÉ

Un modèle général disponible sur micro-ordinateur est décrit; il s'appuie sur la notion de modélisation de la fugacité et contient des expressions pour les processus ou effets suivants:

Biodisponibilité des produits chimiques dans l'eau, compte tenu de la sorption,
Assimilation et clearance par transfert au niveau des branchies,
Consommation d'aliments, digestion et excrétion,
Métabolisme,
Coissance chez les poissons.

Les paramètres du modèle sont analysés et il est démontré que la bioamplification peut se produire par des augmentation successives de concentration à mesure qu'on progresse le long de la chaîne alimentarie. Une "chute" dans la bioaccumulation est prévue avec les produits chimiques très hydrophobes.

Le modèle produit une expression analytique (équation)pour exprimer la concentration en fonction du temps ou, avec les systèmes complexes, une intégration numérique est possible pour l'obtention de résultats graphiques ou en tuyaux d'orgue. On considère que cette formule est particulièrement commode pour "l'ajustement" de données d'expérience sous forme exponentielle pour une série de composés hydrophobes à différents degrés.

AN OVERVIEW OF EPIZOOTIC POLLUTION-RELATED NEOPLASMS IN BONY FISH

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ABSTRACT

Production of synthetic organic chemicals accelerated in the 1930's and has since doubled every 7 to 8 years; with over 60,000 chemicals in common usage and greater than 100,000 chemicals detected in the environment. The historical, epidemiological, experimental and biochemical evidence, as well as lack of a reasonable alternative suggests that fish liver cancer has a chemical etiology. Since liver tumors were first reported in wild fish in 1964, these neoplasms have been reported in a dozen benthic fish species from greater than 20 polluted freshwater and marine waterways. These data, combined with reports of liver neoplasms experimentally produced in 25 species by 35 carcinogens, suggests that fish surveys for liver tumors and tumor induction bioassays have high potential for identification of carcinogenic chemicals in the laboratory and in the field.

RÉSUMÉ

La production des composés organiques synthétiques s'est accélérée dans les années trente et depuis, a doublé tous les 7 ou 8 ans; il y a plus de 60 000 produits chimiques d'usage courant et plus de 100 000 produits chimiques sont trouvés dans le milieu. Les résultats historiques, épidémiologiques, expérimentaux et biochimiques, ainsi que l'inexistence de toute autre explication raisonnable, nous font croire que le cancer du foie chez le poisson a un fondement étiologique chimique. Depuis la première fois que des tumeurs au foie ont été rapportées chez poissons à l'état sauvage, en 1964, ces néoplasmes ont été rapportés chez une douzaine d'espèces de poissons benthiques dans plus d'une vingtaine de grandes voies polluées d'eau douce et d'eau de mer. Lorsqu'on rapproche ces résultats à des rapports concernant la production expérimentale de néoplasmes dans le foie chez 25 espèces par 35 agents carcinogènes, il apparaît que les relevés visant à détecter des tumeurs du foie chez les poissons et que les essais biologiques visant à l'induction de tumeurs offrent beaucoup de potentiel comme instruments servant à l'identification de produits chimiques carcinogènes, au laboratoire et sur le terrain.

POLYNUCLEAR AROMATIC HYDROCARBONS AND TUMORS IN BROWN BULLHEADS FROM THE BLACK AND CUYAHOGA RIVERS - CAUSE AND EFFECT?

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ABSTRACT

Populations of brown bullhead from the Black and Cuyahoga Rivers, two Lake Erie tributaries in Ohio, have elevated tumor frequencies. Epidermal papillomas, squamous carcinomas, and biliary and hepatic liver lesions, including cholangio-and hepatocarcinomas, have been diagnosed in fish from both populations. Grossly observable liver tumors occurred in brown bullhead over 250 mm from the Black River at a frequency of over 30% in 1982 (n=241) and in those from the Cuyahoga River at a frequency of 13% in 1984 (n=90). Randomly selected fish over 250 mm (n=125) from the Black River had a 38% frequency of liver cancer and an 84% frequency of changes in the neoplastic process in 1982. Similarly sized bullhead from the Cuyahoga River (n=21) had a 19% frequency of liver cancer and a 62% frequency of liver changes in the neoplastic process in samples taken in 1984 and 1986. A large number of polynuclear aromatic hydrocarbons (PAHs) occurred in the sediment of both systems. PAH concentrations in the Cuyahoga River ranged from 0.1-1 μg/g dry weight for individual compounds, while these same chemicals occurred at 10-1000 μg/g levels in Black River sediment near the point source. Both systems contained known carcinogens such as benz(a)anthracene and benzo(a)pyrene. Whole brown bullhead form both locations also contained detectable levels of PAHs. Bile samples from Black River bullhead contained elevated levels of PAH metabolites. Organochlorines and elements analyzed were not elevated relative to other systems without epizootics of neoplasia, and preliminary electron microscopy on liver cancers from Black River fish did not find evidence of viral inclusions.

RÉSUMÉ

Les populations de barbotte brune des rivières Black et Cuyahoga, deux tributaries du lac Érié en Ohio, ont une fréquence élevée de tumeurs. Des papillomes épidermiques, des carcinomes squameux et des lésions du foie et du la vésicule biliaire, notamment des cholangio et des hépatocarinomes, ont été diagnostiqués dans les deux populations. Des tumeurs du foie facilement observables étaient trovées chez la barbotte brune de plus de 250 mm qui provenait de la rivère Black à une fréquence supérieure à 30% en 1982 (n=241), et à une fréquence de 13% en 1984 (n=90) chez les spécimens comparables de la rivére Cuyahoga. En 1982, un groupe de poissons de plus de 250 mm (n=125) prélevés au hasard dans la rivère Black avait une fréquence de 38% de cancers du foie et une fréquence de 84% d'altérations du processus néoplastique. Un groupe de barbottes brunes de même taille, mais provenant de la riviére Cuyahoga (n=21), avait une fréquence de 19% de cancers du foie et une fréquence de 62% d'altérations du processus néoplastique au niveau du foie (échantillonnage entre 1984 et 1986). On a trouvé un nombre important d'hydrocarbures (HAP) dans les sédiments des deux réseaux. La concentration de HAP dans la riviére Cuyahoga variait entre 0.1 et 1 μg/g poids sec pour des composés considérés isolément, alors que ces mêmes composés étaient trouvés en concentration de 10 à 1000 μg/g dans les sédiments de la rivière Black près de la source. Les deux systèmes contenaient des

agents carcinogènes connus comme le benz(a)anthracène et le benzo(a)pyrene. Les baarbottes brunes entières des deux rivières contenaient également des HAP en concentration détectable. Les échantillons biliaires de barbottes de la rivière Black contenaient des métabolites de HAP en concentration élevée. Les substances organochlorées et éléments qui ont été dosés n'ont pas été trouvés en concentration élevée relativement à d'autres réseaux où des épizooties d'origine néoplasique n'étaient pas observées; en outre, les examens préliminaires au microscope électronique de tissu cancéreux du foie poisson de la rivière Black.

^{32}P-POSTLABELING DETECTION OF DNA ADDUCTS IN FISH FROM CHEMICALLY CONTAMINATED WATERWAYS

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ABSTRACT

Liver neoplasia has been observed in fish species from a number of polluted waterways. In these areas, the sediments are heavily contaminated by organic chemicals including known carcinogens. In general, bottom dwelling/feeding fish species have had the highest prevalence of neoplasia and thus it has been suggested that exposure to sediment-bound chemical carcinogens may play a role in the development of tumors in these fish. To date, the relationship between chemical contaminants and tumors in fish has been, in general, inferred from fish tumor surveys, chemical analysis of sediments, studies on carcinogen metabolism in fish and laboratory exposures to chemical carcinogens. Detection of DNA adducts in fish would be, in its simplest form, a measure of genetic damage as a result of chemical exposure and may be a highly relevant measure of carcinogenic risk. We have used the sensitive ^{32}P-postlabeling method to analyze carcinogen:DNA adducts in liver tissue from fish collected in polluted waterways. DNA was isolated and hydrolyzed to normal and carcinogen-adducted 3' nucleotides. Hydrophobic/ bulky adducts were isolated by HPLC or treatment with nuclease P1 and then were quantitatively labeled in the 5' position with ^{32}P. Aromatic adducts were selectively isolated and analyzed by TLC on PEI-cellulose. Chromatograms derived from fish from the Buffalo River and Detroit River exhibited a diffuse radioactive zone of putative aromatic carcinogen:DNA adducts not evident in control fish. Total levels of adducts (nM adduct/mole normal nucleotides) in bullheads were 14.8 for control fish, 70.1 for Buffalo River fish and 86 for Detroit River fish. Total levels in white suckers and carp from the Buffalo River were 439 and 430, respectively. These results directly demonstrate chemical interaction with DNA which is generally considered to be the first step in chemical carcinogenesis. Although the specific compounds involved have not been determined, the adducts observed have characteristics that suggest they are hydrophobic and have an aromatic or bulky lipophilic moiety. Adduct analysis conducted along with existing methods for investigating the etiology of tumors in wild fish populations can provide strong evidence linking chemical contaminants and fish tumors.

RÉSUMÉ

Des néoplasmes du foie ont été observés chez différentes espèces de poissons dans nombre de voies d'eau polluées. Dans ces secteurs, les sédiments sont fortement contaminés par des produits chimiques organiques, notamment des carcinogènes connus. En général, les poissons qui vivent et se nourrissent au fond de l'eau ont le taux le plus élevé de néoplasmes et il a donc été suggéré que l'exposition aux carcinogènes d'origine chimique liés aux sédiments pouvait avoir un rôle dans le développement des tumeurs chez ces poissons. Jusqu' à maintenant, le rapport entre les contaminants chimiques et les tumeurs chez les poissons a généralement été déduit de relevés de population pour la découverte de tumeurs, d'analyses chimiques des sédiments, d'études portant sur le métabolisme des carcinogènes chez le poisson et d'expositions en laboratoire à des produits chimiques carcinogènes. La détection de produits d'addition du DNA chez les poissons pourrait, dans sa forme la plus simple, constituer une chimiques et cette détection peut être en étroite corrélation avec les risques de cancer. Nous utilisé la méthode sensible du postmarquage au ^{32}P pour analyser les complexes carcinogènes - produits d'addition du DNA dans le tissu hépatique de poissons échantillonnés dans des voies d'eau polluées. Le DNA a été isolé et hydrolisé jusqu'au niveau des nucléotides normaux et des nucléotides avec produits d'addition carcinogènes en 3'. Les produits d'addition hydrophobes et volumineux ont été isolés par CLHP ou par traitement à la nucléase P1 pour être ensuite marqués quantitativement à la position 5' avec du ^{32}P. Les produits d'addition aromatiques étaient isolés sélectivement et dosés CLT sur PEI-cellulose. Les chromatogrammes correspondant à des poissons capturés dans les rivières Buffalo et Détroit avaient une zone radioactive diffuse constituée de l'agent carcinogène aromatique supposé et des produits d'addition du DNA, qui ne se retrouvait pas chez les témoins. La concentration totale des produits d'addition (nanomoles du produit d'add./mole de nucléotides normaux) chez des barbottes brune était de 14.8 chez les témoins, 70.1 chez les poissons de la rivière Buffalo et 86 chez les poissons de la rivière Détroit. Les concentrations totales chez meunier noir et la carpe de la rivière Buffalo étaient de 439 et 430, respectivement. Ces résultats montrent une interaction chimique directe avec le DNA qui est généralement considérée comme la première étape d'une carcinogènese chimique. Bien que les composés précis n'aient pas été identifiés, les produits d'addition observés avaient certaines caractéristiques indiquant qu'ils sont hydrophobes et ont une fraction, (volumineuse) lipophile ou une fraction aromatique. L'analyses des produits d'addition en même temps que l'application d'autres méthodes connues d'examen de l'étiologie de tumeurs dans des populations sauvages de poissons, peuvent indiquer fortement un lien entre les contaminants chimiques et les tumeurs chez les poissons.

DNA REPAIR AS ASSAYED *IN VIVO*: ITS IMPLICATIONS FOR THE MECHANISMS OF TUMORIGENESIS IN FISH

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ABSTRACT

To throw light on the mechanisms of DNA repair in hitherto non-investigated fish classes, we measured O^6-methylguanine DNA methyltransferase (O^6-MT) activity in 8 species of fresh water fish. In addition, an investigation of the effect of chronic exposure to dimethylnitrosamine on 0^6-MT activity in rainbow trout was included. Further, *in vivo* investigations, including immunological detection of carcinogen-DNA adducts, were directed at revealing the relationship between DNA repair enzyme activity, adduct formation, and subsequent tumor induction.

RÉSUMÉ

Afin d'élucider les mécanismes de la réparation du DNA chez des classes de poisson qui n'ont pas été utilisées jusqu'ici, nous avons mesuré l'activité de la O^6-méthylguanine DNA méthyltransférase (O^6-MT) chez huit espèces de poissons d'eau douce. En outre, il est question d'une étude de l'effet de l'exposition chronique de truites arc-en-ciel à la diméthyl-nitrosamine sur l'activitié de la O^6-MT. De plus, des recherches *in vivo*, notamment la détection immunologique des produits d'addition DNA-carcinogène, ont porté sur le rapport entre l'activité enzymatique liée à la réparation du DNA, la formation de produits d'addition et l'induction subséquente d'une tumeur.

EFFECTS OF SOME POLYNUCLEAR AROMATIC HYDROCARBONS ON SMALL FISH CARCINOGENESIS MODELS

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ABSTRACT

Polynuclear aromatic hydrocarbons (PAHs) have often been implicated in environmental epizootics of cancer in fishes. Indeed, the ability of hepatic microsomes to convert some PAHs to ultimate carcinogenic forms has been demonstrated in numerous fish species. Tumorigenic effects of PAHs in aqueous exposures, however, have rarely been demonstrated in laboratory experiments, possibly because of the poor solubility of those compounds. In our studies, we examined the carcinogenic effects of benzo(a)pyrene (BaP) and 7,12-dimethylbenzanthracene (DMBA) on the Japanese medaka (*Oryzias latipes*) and the guppy (*Poecilia reticulata*). Exposure media were produced by passing PAH suspensions in water with and without dimethylformamide carrier through various sized filters. The low dose was an 0.45 μ filtrate without carrier that contained less than 5 μg/L PAH. The intermediate dose, a carrier-mediated 0.45 μ filtrate, contained 30-50 μg/L PAH; whereas the high dose, a carrier-

mediated glass fiber filtrate, contained 150-250 μg/L PAH. Two 6-h exposures were conducted on young fish specimens. Preliminary analyses indicate that hepatic neoplasms occurred in both medaka and guppy exposed to both BaP and DMBA. In the guppy, BaP-induced hepatic neoplasms were limited to the high exposure group and occurred in incidences of about 10% and 24% at 24 and 36 wk post initial exposure, respectively. DMBA was much more carcinogenic to the fishes than was BaP. In the medaka, each DMBA exposure concentration induced hepatic neoplasms. Incidences in the high and intermediate exposure levels were about 50% at 24 wk. DMBA-induced medaka developed numerous non-hepatic neoplasms as well. These included gastrointestinal neoplasms (10% at 24 wk), renal neoplasms (5% at 24 wk) and single cases of retinal dysplasia and epidermal hemangioma. These studies demonstrate the carcinogenic effect of BaP and DMBA to fishes following brief waterborne exposures in the μg/L range. Furthermore, that hepatic neoplasms in BaP-exposed guppy and medaka and non-hepatic neoplasms in DMBA-exposed medaka were limited to the high exposure concentration suggests that the particulate (insoluble) fraction plays an important role in carcinogenesis. [This study was supported in part by a contract with the American Petroleum Institute.]

RÉSUMÉ

Les hydrocarbures aromatiques polynucléaires (HAP) ont été souvent mis en cause dans les cas d'épizooties du cancer chez les poissons. En effet, on a montré chez nombreuses espèces de poissons que les microsomes du foie peuvent convertir certains HAP en des formes finales qui sont carcinogènes. Les effets oncogènes des HAP administrés dans l'eau ont cependant été rarement établis en laboratoire, peut - être à cause de la mauvaise solubilité de ces composés. Dans nos études, nous avons étudié les effets carcinogènes du benzo(a)pyrène (BaP) et du 7,12-dyméthylbenzanthracène (DMBA) chez *Oryzias latipes* et chez le guppy (*Poecilia reticulata*). Les milieux servant à l'exposition ont été préparés en passant des suspensions de HAP dans l'eau, avec et sans un vecteur constitué de dyméthylformamide, sur des filtres de différents calibres. La dose faible était constituée par le filtrate obtenus sur filtre de 0.45 μ, sans vecteur, qui contenait moins de 5 μg/L PAH. La dose intermédiaire, un filtrat avec obtenu sur filtre de 0.45 μ, contenait 30 à 50 μl/L PAH. La dose élevée était constitutée par un filtrat avec vecteur passé par sur fibre de verre; elle contenait 150 à 250 μg/L PAH. De jeunes spécimens de poissons ont été exposés aux filtrats deux fois pendant 6 heures. Les analyses préliminaires indiquent qu'il s'est formé des néoplasmes hépatiques chez l'*Oryzias* et le guppy exposés au BaP ainsi qu'au DMBA. Sur le guppy, les néoplasmes hépatiques induits par le BaP n'ont été observés que chez le groupe exposé à la plus forte concentration, ceci à la fréquence d'environ 10% et 24%, 24 et 36 semaines après l'exposition initiale, respectivement. Le DMBA s'est révélé être beaucoup plus carcinogène pour les poissons que le BaP. Chez *Oryzias*, toutes les concentrations de DMBA ont donné des néoplasmes hépatiques. Les incidences de néoplasmes aux concentrations élévé et intermédiaire étaient d'environ 50% à 24 semaines. De nombreux néoplasmes non hépatiques sont apparus chez les *Oryzias* exposés au DMBA. Il y avait des néoplasmes gastro-intestinaux (10% à 24 semaines), des néoplasmes rénaux (5% à 24 semaines) et des cas isolés de dysplasie rétinienne et d'angiomes épidermiques. Ces études montrent l'effet carcinogène du BaP et DMBA sur les poissons exposés à ces substances en concentration de l'ordre du μg/L dans l'eu. En outre, ces études ont montré que les néoplasmes hépatiques chez le guppy et *Oryzias* exposés au BaP ainsi que les néoplasmes non hépatiques chez les *Oryzias* exposés au DMBA étaient limités aux cas d'exposition à la forte concentration; cela porte à penser que la fraction des par-

ticules (insoluble) a un rôle important dans la carcinogènese. (Cette étude a été subventionnée en partie par l'*American Petroleum Institute*.)

HISTOPATHOLOGY OF FERAL AND EXPERIMENTAL WINTER FLOUNDER EXPOSED TO CONTAMINATED HARBOUR SEDIMENTS

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ABSTRACT

Pathological effects in winter flounder (*Pseudopleuronectes americanus*) were investigated using a chemically polluted sediment from Black Rock Harbor (BRH), CT, USA. Substances contained in BRH sediment are known to be genotoxic and carcinogenic. In experimentally exposed winter flounder, proliferative lesions were observed in external, oral and esophageal epithelia, renal vascular and nephroblastic elements, and the pancreatic islets. Islet lesions included cystic adenomas and a diffuse islet hyperplasia in ductal mucosa, nesidioblastosis. In addition, trophic transfer of BRH sediment via mussels (*Mytilus edulis*) appeared to enhance pathology. Feral winter flounder were collected to determine the prevalence of neoplastic and other pathologic disorders. Collections were conducted in Black Rock Harbor per se, in Long Island Sound at a site selected for disposal of dredged BRH sediment and at a reference location in Narragansett Bay, RI.

RÉSUMÉ

Des sédiments pollués par des produits chimiques et provenant de Black Rock Harbor (BRH), CT, USA, ont été utilisés pour leurs effets pathologiques chez la plie rouge (*Pseudopleuronectes americanus*). Les substances contenues dans les sédiments de BRH sont connues pour leur toxicité sur le plan génétique et sur le plan carcinogène. Chez des plies rouges exposées expérimentalement, des lésions prolifératives ont été observées au niveau des épithéliums de la peau, de la bouche et de l'oesophage, au niveau des éléments néphroblastiques et vasculaires rénaux ainsi qu'au niveau des ilots de Langerhans. Á ce dernier niveau, on a observé des adénomes kystiques et une hyperplasie diffuse dans les muqueuses des canaux ainsi que des tumeurs insularies du pancréas. En outre, le passage d'un niveau trophique à l'autre des sédiments BHR par l'intermédiaire des moules (*Mytilus edulis*) semble avoir accentué la pathologie. Des plies rouges sauvages ont été capturées afin de déterminer la fréquence d'apparition de néoplasmes et d'autres troubles pathologiques. Des échantillonnages ont été faits à Black Rock Harbor même, dans le détroit de Long Island, à l'endroit choisi pour le rejet des sédiments dragués de BRH et à une station de référence à Narrangansett Bay, RI.

STUDIES ON LIVER CARCINOGENESIS IN ENGLISH SOLE FROM PUGET SOUND, WASHINGTON, USA: I. PATHOLOGIC ANATOMY AND PATTERNS OF OCCURRENCE OF NEOPLASMS, PRENEOPLASTIC FOCAL LESIONS AND OTHER IDIOPATHIC HEPATIC CONDITIONS; EVIDENCE FOR A XENOBIOTIC CHEMICAL ETIOLOGY

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ABSTRACT

Livers of feral English sole (*Parophrys vetulus*) from polluted embayments of Puget Sound are affected by a spectrum of multiple, co-occurring idiopathic hepatic lesions, including neoplasms, preneoplastic foci of cellular alteration and unique degenerative conditions. Results from a statistical analysis of the patterns of co-occurrence of these lesions in feral English sole are consistent with the following concept. These are morphologically identifiable steps strongly suggesting a progressive sequence toward hepatic neoplasms in English sole that parallel the types of lesions and sequence of lesion progression in experimental models of chemically induced liver carcinogenesis in rodents. The rationale for the hypothesis that these lesions in feral English sole may be caused by exposure to certain xenobiotic hepatotoxic and hepatocarcinogenic compounds is also based on the demonstration of: (1) significant associations between levels of aromatic hydrocarbons in the sediment and prevalence of these idiopathic liver lesions; (2) uptake, metabolism and disposition of potentially hepatotoxic/hepatocarcinogenic aromatic hydrocarbons by English sole (see Stein et al. in this volume); and (3) significant associations between levels of fluorescent metabolites of aromatic compounds in bile of English sole and the prevalence of neoplasms and other idiopathic liver lesions.

Relevant data from ongoing experimental exposures of English sole and other fish species to sediment extracts containing various mixtures of environmental contaminants and to model hepatocarcinogens will be discussed.

RÉSUMÉ

Le foie de soles anglaises sauvages (*Parophrys vetulus*) capturées dans des baies polluées de la région de Puget Sound porte toute une série de lésions idiopathiques, multiples et concomitantes, notamment des néoplasmes, des foyers prénéoplastiques d'altération cellulaire et des conditions dégénératives particulières. Les résultats d'une analyse statistique des formes de concomitance de ces lésions chez cette espèce à l'état sauvage confirment le concept suivant : ce sont des étapes indentifiables sur le plan morphologique qui indiquent nettement une séquence progressive d'événements conduisant à des néoplasmes hépatiques chez la sole anglaise et qui sont semblables au type de lésions et à la séquence de progression des lésions obtenus par des modèles expérimentaux de carcinogènese du foie induite chimiquement chez les rongeurs. Les raisons de proposer cette hypothèse à l'effet que les lésions chez les soles anglaises sauvages peuvent être causées par une exposition à certains composés xénobiotiques hépatotoxiques et hépatocarcinogènes, tiennent aussi à l'établissement : 1) d'associations significatives entre les concentrations d'hydrocarbures aromatiques dans les sédiments et la prévalence de ces lésions hépatiques idiopathiques; 2) de l'absorption, du métabolisme et de l'élimination d'hydrocarbures aromatiques potentiel-

lement hépatotoxiques/hépatocarcinogènes chez la sole anglaise (voir Stein et al., ce volume); 3) d' associations significatives entre les concentrations de métabolites fluorescents de composés aromatiques dans la bile de la sole anglaise ainsi que la prévalence de néoplasmes et d'autres lésions idiopathiques du foie.
Il sera question des résultats d'expositions expérimentales permanentes de sole anglaise et d'autres poissons à des extraits de sédiments contenant différents mélanges de contaminants trouvés dans le milieu à des hépatocarcinogènes types.

STUDIES ON LIVER CARCINOGENESIS IN ENGLISH SOLE FROM PUGET SOUND, WASHINGTON, USA: II. UPTAKE, ACTIVATION AND DETOXICATION OF POLYCYCLIC AROMATIC HYDROCARBONS

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ABSTRACT

The levels of aromatic hydrocarbons in sediments in Puget Sound, WA, are positively correlated with the prevalence of hepatic neoplasms in English sole (*Parophrys vetulus*), and the types of lesions and sequence of lesion progression in sole are consistent with studies on chemically-induced liver carcinogenesis (see Myers et al. in this volume). To investigate biochemical processes involved in chemical carcinogenesis in English sole from Puget Sound, we have studied the uptake, activation and detoxication of polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene (BaP), by this species. The results show that sediment-associated PAHs are bioavailable to sole, and that the presence of other xenobiotics in sediment influences the extent of uptake and metabolism of BaP. The ability of English sole to metabolize PAHs to reactive intermediates that bind to DNA was investigated using the ^{32}P-postlabeling technique. Analysis of hepatic DNA from sole sampled from urban sediments indicated that PAHs detected in urban sediments are taken up by sole and metabolized to intermediates that covalently bind to DNA. Further, comparison of the activation and detoxication of BaP by sole and a related species, starry flounder (*Platichthys stellatus*), which shows a lower prevalence of hepatic neoplasms than sole when sampled from urban area, indicated that detoxication of reactive metabolites may be an important factor in the apparently higher susceptibility of English sole to PAH-induced carcinogenesis.

RÉSUMÉ

Il existe une corrélation positive entre la concentration d'hydrocarbures aromatiques dans les sédiments de Puget Sound, WA, et la prévalence de néoplasmes hépathiques chez la sole anglaise (*Parophrys vetulus*) les types de lésions ainsi que la séquence de progression des lésions chez la sole concordent avec les résultats d'études sur la carcinogenèse du foie induite chimiquement (Myers et al., ce volume). Pour comprendre les processus biochimiques en cause dans la carcinogenèse chimique chez la sole anglaise de Puget Sound, nous nous appuyons sur des études d'absorption, d'activation et de de'toxication liées aux hydrocarbures aromatiques polycycliques (HAP)

comme le benzo(a)pyrène (BaP), chez cette espèce. Les résultats montrent que les HAP liés aux sédiments peuvent être assimilés par la sole; en outre, la présence d'autres substances xénobiotiques dans les sédiments agit sur le degré d'assimilation et de métabolisme du BaP. La capacité de la sole anglaise à métaboliser les HAP en intermédiaires réactifs qui se combinent au DNA a fait l'objet d'études avec la technique de post-marquage au ^{32}P. L'analyse du DNA hépatique chez les soles capturées en secteurs de sédiments urbains a indiqué que les HAP détectés dans ces sédiments sont assimilés par la sole et métabolisés en composés intermédiaires qui se combinent par covalence au DNA. En outre, la comparaison de l'activation et de la détoxication dans le cas du BaP chez la sole et chez une espèce apparentée, la plie étoilée (*Platichthys stellatus*), chez qui il y a moindre prévalence de néoplasmes hépatiques que chez la sole parni les sujets échantillonnés en milieu urbain, cette comparaison indique que la détoxication par l'elimination de métabolites réactifs peut constituer un facteur important de la susceptibilité apparemment forte de la sole anglaise à la carcinogenèse induite par les HAP.

EXPERIMENTAL INDUCTION OF LIVER TUMORS IN RAINBOW TROUT WITH EXTRACTS FROM CONTAMINATED SEDIMENTS

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ABSTRACT

In order to investigate the putative association between chemical contaminants and high incidences of fish tumors in western Lake Ontario, sediments from Hamilton Harbour, Oakville Creek, and a control site in Georgian Bay (South Baymouth) were collected and extracted for organic contaminants. Sediment extract from Hamilton Harbour had the highest levels of PCBs, organochlorine pesticides, and polynuclear aromatic hydrocarbons. This extract was also mutagenic in the Ames mutagenicity assay. In two sets of experiments using the trout embryo micro-injection assay, Hamilton Harbour sediment extract induced hepatocellular carcinomas in rainbow trout. In addition to these tumor initiation studies, sediment extracts are also being tested for promotional activity.

RÉSUMÉ

Afin d'examiner le rapport supposé entre des contaminants chimiques et une incidence élevée de tumeurs chez les poissons dans la partie ouest du lac Ontario, des sédiments du port d'Hamilton, d'Oakville Creek et d'une station témoin de la baie Georgienne (South Baymouth) ont été prélevés et les contaminants organiques qu'ils contenaient ont été extraits. Les extraits du port d'Hamilton contenaient le plus de PCB, de pesticides organochlorés et d'hydrocarbures aromatiques polynucléaires. Cet extrait était également mutagène d'après le test de mutagénicité d'Ames. Dans deux séries d'expériences de microinjection dans les embryons de truite, on a observé que l'extrait des sédiments du port d'Hamilton induisait des carcinomes hépatocellulaires

chez la truite arc-en-ciel. En plus de ces études de formation de tumeurs, le potentiel comme activateur des extraits est également testé.

THE OCCURRENCE OF EPIDERMAL PAPILLOMAS AND LIVER NEOPLASIA IN WHITE SUCKERS (*CATOSTOMUS COMMERSONI*) FROM LAKE ONTARIO

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ABSTRACT

White suckers from Lake Ontario are affected with epidermal lesions occurring on the lips and bodies of mature fish. The majority of tumours are benign papillomas but a small number are squamous cell carcinomas and dermal fibrosarcomas. Surveys conducted in 1973-75 reported increased tumour prevalence in suckers from western Lake Ontario and suggested a relationship between papillomas and urban development. These studies were expanded between 1981-87 to determine variability between sites, temporal trends, and the utility of papillomas as indicators of deteriorating environments.

Hamilton Harbour spawning populations were monitored from 1981-1983. The prevalence of lip papillomas in 1981-83 (39%) was similar to that reported in 1973-75 (35%). Papilloma frequency increased with age and was independent of sex. Fish with single and multiple papillomas contributed to the spawning population. There were no adverse effects on migratory behaviour, fecundity, or embryo development. Tag returns indicated that feral white suckers retained their lip papillomas for at least one year and that large multiple papillomas could develop rapidly. Tumour prevalence was monitored at 13 sites on Lake Ontario and six sites on Lake Huron in 1982-83. The prevalence of lip papillomas ranged from 5% on the Bay of Quinte to 62% at Sixteen Mile Creek and from 0-39% on Lake Huron. Body lesions were less frequent and ranged from 0-39% on Lake Huron. Further sampling in 1984-85 at Hamilton Harbour, Sixteen Mile Creek, and a control site on Manitoulin Island indicated the presence of cholangiolar carcinomas at all sites (1.7%, 6.5% and 3.7% respectively). In addition, fish from Hamilton Harbour and Sixteen Mile Creek were affected with hepatocellular tumours (0.9% and 2.8%) and adenomas (0.9% and 2.2%). There were no hepatocellular tumours at the control site. Four additional sites along the north shore of Lake Ontario were sampled in 1987.

There was no apparent relationship between liver tumours and PAHs in sediments from Hamilton Harbour, Sixteen Mile Creek, and the control site. However, chemical exposure patterns are complicated by fish movement. White sucker distribution in Hamilton Harbour is influenced by the availability of food and suitable habitat and fish rarely inhabit the most contaminated areas. In addition, suckers are temporary residents of the harbour and spend part of the year in Lake Ontario. Movement in the lake is unknown but one fish was recaptured 60 km from the spawning site. Tumour induction studies using extracts from the three sites are underway.

RÉSUMÉ

Les meuniers noirs du lac Ontario souffrent de lésions épidermiques qui apparaissent sur les lèvres et la peau des poissons adultes. La majorité des tumeurs est constituée de papillomes bénins, mais un petit nombre des tumeurs sont des carcinomes des cellules squameuses et des fibrosarcomes dermiques. Les relevés effectués en 1973-1975 ont indiqué une prévalence accure de tumeurs chez le meuniers de la partie ouest du lac Ontario; ceci indique un rapport entre les papillomes et l'urbanisation. Entre 1981 et 1987, ces études ont été élargies de façon à ce qu'on puisse déterminer la variabilité entre les stations, les tendances dans le temps et l'utilité des papillomes comme indicateurs des la détérioration du milieu.

Les populations reproductrices du port d'Hamilton ont été étudiées entre 1981 et 1983. La prévalence de papillomes sur la lèvre en 1981-1983 (39%) était semblable à ce qu'elle était en 1973-1975 (35%). La fréquence des papillomes augmentait avec l'âge et n'avait aucun rapport avec le sexe. Les poissons qui avaient un papillome ou des papillomes multiples participaient à la reproduction. Il n'y avait pas d'effet négatif sur le comportement migrateur, la fécondité ou le développement des embryons. Les retours de bagues ont indiqué que les meuniers noirs sauvages portaient ces papillomes des lèvres pendant au moins un an et que les gros papillomes multiples pouvaient apparaître rapidement. La prévalence de tumeurs a été mesurée à 13 stations du lac Ontario et six du lac Huron en 1982-1983. La prévalence des papillomes des lèvres variait entre 5% à la baie de Quinte, et 62% à Sixteen Mile Creek, et de 0 à 39% dans le lac Huron. Les lésions ailleurs sur le corps, moins fréquentes, variaient entre 0 et 39% chez sujets du lac Huron. De nouveaux échantillonnages fait en 1984-1985 au port d'Hamilron, à Sixteen Mile Creek et dans une station témoin de l'île Manitoulin, ont indiqué qu'il y avait des sujets atteints de carcinomes de type choloangiomes dans toutes les stations (1.7%, 6.5% et 3.7%, respectivement). En outre, les poissons du port d'Hamilton et de Sixteen Mile Creek avaient des tumeurs hépatocellulaires (0.9% et 2.8%) ainsi que des adénomes (0.9% et 2.2%). Aucun cas de tumeur hépatocellulaire n'a été observé à la station témoin. Quatre autres stations le long de la rive nord du lac Ontario ont fait l'objet d'échantillonnages en 1987.

Il n'existe pas de rapport apparent entre les temeurs du foie et les HAP des sédiments du port d'Hamilton, de Sixteen Mile Creek et de la station témoin. Cependant, la configuration des expositions à des agents chimiques se trouve compliquée par les mouvements des poissons. La distribution du meunier noir dans le port d' Hamilton est influencée par disponibilité des aliments et l'acceptabilité de l'habitat; or le poisson habite rarement dans les eaux contaminées. En outre, les meuniers sont des résidents temporaires du port d'Hamilton et passent une partie de l'année dans le lac Ontario. Nous ignorons les déplacements de ces poissons dans le lac, mais un d'entre eux a été capturé à 60 km de sa frayère. Il y a des études en cours sur l'induction de tumeurs avec les trois extraits provenant des trois stations.

PATHOGENESIS OF SKIN AND LIVER NEOPLASMS IN WHITE SUCKERS (*CATOSTOMUS COMMERSONI*) FROM POLLUTED AREAS IN LAKE ONTARIO

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ABSTRACT

Increased incidences of epidermal and hepatobiliary neoplasms in white suckers in Lake Ontario have been associated with industrial pollution, but the causative agents have not been identified. We have recognized that the vast majority of epidermal tumors of lip and body skin are benign focal proliferations. These skin tumors do not have consistent alterations in glutathione (GSH)-dependent detoxification enzymes, including GSH-peroxidase (GPO), GSH reductase (GR), gamma-glutamyltranspeptidase (GGT) or glutathione-S-transferase (GST) isoenzymes, suggesting that their growth is not promoted by toxic chemical agents. Most epidermal lesions disappear when fish are maintained in captivity, but new lesions develop in these "clean" conditions, further suggesting that environmental xenobiotics are not involved in the promotional phase of development of these skin tumors. By comparison, liver tumors include preneoplastic, benign and malignant variants of hepatocellular and biliary types, the latter being more frequent and consistently associated with chronic proliferative cholangiohepatitis. The appearance of phenotypically altered populations to hepatocytes in these livers suggests prior exposure to genotoxic initiating carcinogens. The chronic biliary disease may reflect either an infectious disease or a toxic injury caused by xenobiotics concentrated in bile during hepatic metabolism and excretion. The alterations in hepatic GST isoenzymes of suckers and other species of fish from clean and polluted areas have been evaluated as a means of detecting chemically induced preneoplastic hepatocellular and biliary lesions. Techniques for evaluating the role of GSTs in limiting carcinogen injury in monolayer hepatocyte culture are being developed to facilitate bioassays for environmentally significant carcinogens that are putatively involved in cholangiohepatitis and liver neoplasms of white suckers. These studies are necessary to estimate the risk to humans exposed to environmental xenobiotics that contribute to the pathogenesis of neoplasms in wild fish.

RÉSUMÉ

L'incidence accrue de néoplasmes hépato-biliaires chez le meunier noir du lac Ontario a été attribuée à la pollution industrielle, mais les agents à l'origine de ce phénomene n'ont pas été identifiés. Nous savons maintenant que la grande majorité des tumeurs épidermiques (au niveau des lèvres et de la peau) sont des proliférations bénignes en foyer. Il n'y a pas, associées à ces tumeurs de la peau, d'altérations des enzymes de la détoxication dépendantes du glutathion (GSH), notamment la GSH-peroxydase (GPO), la GSH réductase (GR), la gammaglutamyltranspeptidase (GGT) ou les isoenzymes de la glutathion-S-transférase (GST); cela semble indiquer que la croissance des tumeurs n'est pas activée par des agents chimiques toxiques. La plupart des lésions épidermiques disparaissent quand les poissons sont gardés en captivité, mais de nouvelles lésions apparaissent dans ce milieu "salubre", ce qui nous porte davantage à penser que les composés xénobiotiques dans le milieu n'activent pas le développement de ces tumeurs épidermiques. Par comparaison, on obser parmi le types de tumeurs du foie des variantes prénéoplastiques, bénignes et malign

des types hépatocellulaire et biliaire; la dernière forme mentionnée est la plus fréquente et elle est régulièrement associée à la cholangiohépatite proliférative chronique. L'apparition de populations d'hépatocytes au phénotype modifié dans les foies atteints semble indiquer une exposition préalable à des composés carcinogènes à effets génotoxiques. La bilharioze chronique peut être symptomatique d'une maladie infectieuse ou d'une lésion toxique produites par la concentration de composés xénobiotiques dans la bile résultant du métabolisme et de l'excrétion au niveau du foie. On a évalué l'altération des isoenzymes GST hépatiques de meuniers et d'autres espèces de poissons provenant de secteurs pollués ou non comme moyen de détection des lésions biliaires et hépatocellulaires prénéoplastiques chimiquement induites.
On s'occupe de la mise au point de techniques d'évaluation de l'action des GST pour limiter les dégâts causés par des carcinogènes avec des cultures d'hépatocytes en couche monocellulaire, cela afin de faciliter la tenue d'essais biologiques axés sur les principaux agents carcinogènes du milieu dont on pense qu'ils interviennent dans la choloangiohépatite et les néoplasmes du foie chez le meunier noir. Ces études sont nécessaires à l'estimation du risque couru par les humains exposés à des substances xénobiotiques dans le milieu qui participent à la pathogenèse de néoplasmes chez des populations sauvage de poissons.

THE UNIVERSITY OF TORONTO CULTURE COLLECTION (UTCC)

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ABSTRACT

Cultures of algae and cyanobacteria for environmental research e.g. bioassay, toxicity testing are now available at the University of Toronto. The University of Toronto Culture Collection (UTCC) was recently set up in the Department of Botany with logistic support from the Institute for Environmental Studies, under the administration of Drs. P.M. Stokes, J.R. Coleman and C. Nalewajko. One of its main goals is to supply high quality pure cultures of microorganisms (algae, cyanobacteria, selected bacteria) to the research community in Canada, particularly for environmental science and environmental engineering. At present about 140 isolates are being cultured and more are constantly being added. Currently, most of these are freshwater microalgae and cyanobacteria, some of which have been isolated from extreme environments e.g. metal polluted sites (*Euglena mutabilis*, *Chlamydomonas acidophila* from the Sudbury area and *Chlorella vulgaris* from the Yukon). Some of the standard bioassay test organisms are being cultured, e.g. *Selenastrum capricornutum* and *Scenedesmus acutus*. Cu and Ni tolerant strains of *S. acutus* are also available. Cultures are supplied to researchers for a fee of $30 each to non-profit organizations and $80 each to commercial organizations. Further information and a species list can be obtained from the curator, J. Acreman.

RÉSUMÉ

Des cultures d'algues et de cyanobactéries pour fins de recherche écologique, p. ex., des essais biologiques et des tests de toxicité, sont maintenant disponibles à l'univer-

sité de Toronto. Cette collection de cultures a été récemment constituée par le département de botanique avec l'appui logistique de l'Institut pour l'étude de l'environnement sous l'administration de MMe. P.M. Stokes, J.R. Coleman et C. Nalewajko. L'un des grands objectifs visés est d'offrir des souches pures et de grand qualité de microorganismes (algues, cyanobactéries, certaines souches bactériennes) aux chercheurs canadiens, notamment ceux qui s'occupent d'écologie et de génie de l'environnement. Á l'heure actuelle, environ 140 souches constituent la collection et la liste s'allonge constamment. Pour l'instant, la plupart sont des algues microscopiques et des cyanobactéries d'eau douce, dont certaines proviennent d'environnements extrêmes, p. ex., des endroits fortement pollués par les métaux (*Euglena mutabilis*, *Chlamydomonas acidophila* de la région de Sudbury et *Chlorella Vulgaris* du Yukon). Certains organismes servant aux essais biologiques standards sont maintenant disponibles, p. ex., *Selenastrum capricornutum* et *Scenedesmus acutus*. Des souches tolérantes au Cu et au Ni de *S. acutus* sont également disponibles. Les cultures sont offertes aux chercheurs au service d'organismes à but non lucratif pour la somme 30$ chacune, et pour la somme de 80$ chacune aux sociétés à but lucratif. Pour plus de renseignements et pour la liste des espèces, s'adresser au conservateur J. Acreman.

DIFFERENCES IN CRAYFISH MERCURY RELATED TO SPECIES AND SITES, FIELD RESULTS AND PLANNING OF A FIELD EXPERIMENT

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ABSTRACT

Fourteen lakes from south-central Ontario, with alkalinity and fish Hg gradients were surveyed for crayfish between June and August, 1986. Crayfish abdominal muscles were analyzed for Hg. Significant relationships between Hg concentrations and crayfish total weight were found in five crayfish species (*Cambarus bartoni*, *Cambarus robustus*, *Orconectes virilis*, *Orconectes propinquus*, *Orconectes obscurus*). Bioaccumulation of Hg was faster in the smallest species (*O. propinquus*) than in any other species. In Lake Vernon and Little Hawk Lake, where both lake and stream were sampled, we found stream crayfish (*C. bartoni*) had significantly higher Hg than lake crayfish. There was positive correlation between crayfish and fish Hg. Alkalinity and dissolved organic carbon explained 70% of the variance found in crayfish Hg in the different lakes. We think that part of the Hg found in crayfish is coming from the water. Details of a field transplant experiment done in 1987, are given.

RÉSUMÉ

Des écrevisses ont été échantillonnées entre les mois de juin et d'août 1986 dans quatorze lacs du centre-sud de l'Ontario présentant des gradients d'alcalinité et de Hg dans les poissons. Le muscle abdominal des écrevisses a été analysé afin d'en déterminer la concentration en Hg. Nous avons trouvé qu'il existe une corrélation positive entre le poids total et la concentration en Hg chez cinq espèces d'écrevisses (*Cambarus bartoni*, *Cambarus robustus*, *Orconectes virilis*, *Orconectes propinquus*, *Orconectes obscurus*). La bioaccumulation du Hg s'effectue plus rapidement chez la

plus petite espèce (*O. propinquus*) que chez aucune autre espèce d'écrevisse. Dans les lacs Vernon et Little Hawk, où lac et ruisseau ont été échantillonnés, nous avons trouvé que les écrevisses (*C. bartoni*) récoltées dans les ruisseaux présentaient des concentrations en Hg beaucoup plus élevées que les écrevisses récoltées dans le lac. Il existe une corrélation positive entre les concentrations de Hg des écrevisses et celles des poissons. Les valeurs d'alcalinité et de carbone organique dissout dans l'eau de ces lacs réussissent à expliquer près de 70% de la variance trouvée dans les concentrations de Hg dans les écrevisses. Nous pensons qu'une bonne partie du Hg que l'on trouve dans les écrevisses pourrait venir de l'eau. Nous présentons le plan d'une expérience de terrain effectuée en 1987.

A TEST OF THE ABILITY OF STANDARD, SINGLE-SPECIES, BENCH-TOP BIOASSAYS TO PREDICT ECOSYSTEM SENSITIVITY TO A TOXICANT

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ABSTRACT

Substantial concern has been expressed over the validity of using laboratory derived toxicity data to set water quality objectives or criteria for the protection of aquatic ecosystems. The sensitivity of the ecosystem could be greater than that observed in single species toxicity tests if species interactions are affected at lower toxicant levels than the parameters measured in the tests. Alternatively, the ecosystem may be less sensitive than predicted if the system possesses compensatory mechanisms. We determined the response of a laboratory planktonic mesocosm containing daphnia and phytoplankton to Cd stress, and compared this to the results of a standard bench-top bioassay. Cd concentrations of 5 μg/L and above completely destroyed the stability of the ecosystems. At concentrations of 1 μg/L and below, the ecosystem appeared to remain stable. A Cd concentration of 2 or 4 μg/L significantly reduced the number of young produced by daphnia in 3 wk bench-top bioassays. Comparison of ecosystem response with bench-top bioassay indicated that the bioassay could be used to accurately predict the concentration of Cd below which no detrimental ecosystem effects are likely to occur. However, the degree of response of the ecosystem to sublethal toxic concentrations was much greater than expected. Although the daphnia responded positively to increases in phytoplankton at low phytoplankton levels, very high algal biomass inhibited daphnia population growth. Sublethal Cd levels in the ecosystem resulted in an increase in phytoplankton levels which then acted synergistically to further reduce daphnia population biomass, ultimately resulting in the virtual elimination of the daphnia.

RÉSUMÉ

Beaucoup de spécialistes se soucient de la validité de la démarche consistant à utiliser les résultats de tests de toxicité obtenus en laboratoire pour fixer des objectifs ou des critères de qualité de l'eau visant à la protection des écosystèmes aquatiques. La sensibilité d'un écosystème pourrait être supérieure à ce qui est observé

lors de tests de toxicité sur des espèces uniques lorsque les interactions entre espèces sont modifiées par substances toxiques en concentration moindre que celle utilisée pour l'examen des paramètres dans les tests. Á l'inverse, l'écosystème peut se révéler moins sensible qu'il n'était prévu lorsque des mécanismes de compensation interviennent. Nous avons déterminé en laboratoire la réaction d'une mésocosme planctonique stressé par le Cd qui était constitué notamment de daphnées et de phytoplancton; nous avons comparé nos résultats aux résultats d'un essai biologique standard en laboratoire. En concentration de 5 µg/L et plus, le Cd détruisait la stabilité des écosystèmes. Á 1 µg/L et moins, l'écosystème paraissait rester stable. Une concentration de 2 ou 4 µg/L réduisait significativement la descendance de *Daphnia* lors d'essais biologiques en laboratoire de trois semaines. La comparaison des réactions de l'écosystème et des résultats obtenus avec l'essai biologique en laboratoire , fait ressortir que l'essai biologique pouvait prévoir avec précision jusqu'à quelle concentration aucun effet nuisible sur l'écosystème n'est probable. Cependant, le degré de réaction de l'écosystème à des concentration toxiques sublétales était bien supérieur à ce qui était attendu. Bien que *Daphnia* ait réagi positivement à l'abondance accrue de phytoplancton à de faibles concentrations de ce dernier, la biomasse très élevée d'algues inhibait la multiplication de la daphnée. Les concentrations sublétales de cadmium dans l'écosystème ont conduit à un relèvement de la concentration du phytoplancton est ce phénomène a agi en synergie pour réduire davantage la biomasse de daphnées, ceci a conduit ultimement à l'élimination virtuelle de la daphnée.

RESPONSE OF THE MUSSEL *ANADONTA GRANDI* TO ACID AND ALUMINUM. COMPARISON OF BLOOD IONS FROM LABORATORY AND FIELD RESULTS

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ABSTRACT

Alum (aluminum sulphate) was added to 21-ha Lake 114 (pH 5.9) in the Precambrian Shield at the Experimental Lakes Area (ELA) during the summer of 1984 to observe chemical and biological effects. Mussels were introduced from a second lake, 377, in cages at five sites, five days prior to the three-day alum addition. Effects of exposure to pH 5.9 were elevated blood [Ca^{++}], depressed [Mg^{++}] and temporary increase in [Cl^{-}]. Mussels nearest the site of alum addition experienced a short-term exposure to pH 4.5 and 2237 µg/L total lumogallion-reactive Al. Blood [Ca^{++}] was elevated further and [Na^{+}] and [Cl^{-}] declined. Mussels suffered 0.8% mortality during 26 d in Lake 114.

To separate the effects of acid and Al on blood ions, mussels were exposed in the laboratory to six acid (pH 7.0, 5.0, 4.5) - Al (0, 500 µg/L) combination, each prepared with ELA lake water. Unexpectedly, after transport from ELA to Winnipeg and residence of one d in the laboratory in ELA lake water, mussels had blood [Na^{+}], [K^{+}], [Mg^{++}], [Cl^{-}] and [SO_4^{--}] significantly lower and [Ca^{++}] higher than in blood

freshly collected in the field. No recovery to field concentrations was observed. Al at pH 7.0 had no further effect on blood ions over 9-d exposure but acid (pH 4.5) significantly depressed [Na^+] and elevated [Ca^{++}] further. Al at pH 4.5 exacerbated the effect by still further reducing [Na^+] and [Cl^-] and elevating [Ca^{++}].

In this case, laboratory experiments were useful *qualitatively* in separating the effects of acid and Al on blood ions but the blood ion concentrations measured in the laboratory did not *quantitatively* reflect those in field populations. The mussels were stressed by the laboratory conditions.

RÉSUMÉ

De l'alun (sulfate d'aluminium) a été déversé dans le lac 114 pH 5.9 et 21 ha du superficie) situé dans le Bouclier précambrien de la région des lacs expériment aux (RLE), durant l'été 1984, afin d'observer les effets chimiques et biologiques. Des moules provenant du lac 377 ont été placées dans les cages à cinq endroits dans le lac, cinq jours avant le traitement qui consistait en l'addition d'alun en trois jours. Les effets de l'exposition au pH 5.9 ont été une élévation de [Ca^{++}], une diminution de [Mg^{++}] st une augmentation temporaire de [Cl^-] dans le sang. Les moules situées le plus près du point de déversement de l'alun ont été brièvement exposées à une eau de pH 4.5 et contenant 2237 µg/L d'Al total réactif au lumogallion. La [Ca^{++}] sanguine s'est élevée davantage et la [Na^+] ainsi que la [Cl^-] ont diminué. Il y a eu 0.8% de mortalité chez les moules en 26 jours dans le lac 114.

Afin de distinguer les effets du pH acide de ceux de l'Al sur les ions sanguins, des moules ont été exposées au laboratoire à six combinaisons de pH (pH 7.0, 5.0, 4.5) et de concentration d'Al (0, 500 µg/L), toutes solutions préparés avec de l'eau des lacs de la RLE. Un des résultats imprévus, c'est qu'après le transport des poissons jusqu'à Winnipeg et le séjour d'une journée au laboratoire dans l'eau des lacs de la RLE, la concentration en Na^+, K^+, Mg^{++}, Cl^- et SO_4^{-} était significativement inférieure, alors que celle du Ca^{++} était supérieure aux concentrations sanguines observées sur le terrain chez des moules fraîchement ceuillies. Les moules rapportées au laboratoire n'ont jamais rejoint les concentration observées sur le terrain. Á pH 7.0, l'aluminium n'avait pas d'autre effet sur les ions sanguins après exposition de neuf jours, alors que le pH acide (pH 4.5) a accentué la baisse de [Na^+] et l'augmentation de la [Ca^{++}] de façon significative. Á pH 4.5, l'aluminium a accentué cet effet en réduisant davantage la [Na^+] et en élevant la Ca^{++}.

Dans ce cas-ci, les expériences en laboratoire ont servi *qualitativement* à distinguer les effets du pH acide de ceux de la concentration en Al sur les ions sanguins, mais les concentrations en ions sanguins mesurées au laboratoire n'ont pas reflété *quantitativement* celles observées sue le terrain. Les moules étaient stressées par les conditions de laboratoire.

EXTENDED ABSTRACT

Aluminum frequently increases in concentration in fresh waters which have become acidified. The acidity appears to increase the toxicity of aluminum to aquatic organisms. In order to test the hypothesis that low pH, episodic events cause stress and mortality in aquatic organisms, including mussels, alum (aluminum sulphate) was added to 21-ha Lake 114 in the Experimental Lakes Area (ELA) during the summer of

1984 (Playle, 1987). Our study examined the effects of alum addition on ionic concentrations in the blood of the floater mussel, *Anodonta grandis*.

Mussels were introduced into Lake 114 from a second lake, 377, in cages at five sites five days prior to the three-day alum addition. Mussels responded to the ambient lake pH of 5.9 before the alum addition with elevated blood [Ca^{++}], depressed [Mg^{++}] and temporary increase in [Cl^-]. Mussels nearest the site of alum addition experienced a short-term exposure to pH 4.5 and 2237 ug/L total lumogallion-reactive Al. In these mussels blood [Ca^{++}] was elevated further and [Na^+] and [Cl^-] declined. Mussels suffered 0.8% mortality during 26 days in Lake 114 (Malley et al. in press).

The separate effects of acid and Al on blood ions were studied by transporting mussels from ELA to the Freshwater Institute (FWI) and exposing them to four acid (pH 7.0, 4.5) - Al (0, 500 ug/L) combinations, each prepared with ELA lake water. The effect on blood ions of maintaining mussels for 10 days in the laboratory in ELA lake water was also noted.

Unexpectedly, after transport from ELA to the FWI laboratory and residence of one day in ELA lake water, mussels had blood [Na^+], [K^+], [Mg^{++}], [Cl^-] and [$SO_4^=$] significantly lower and [Ca^{++}] higher than in blood freshly collected in the field (Fig. 1). No recovery to field concentrations was observed during a further 9 day period (Fig. 2).

Aluminum at pH 7.0 had no further effect on most blood ions over the 9-day exposure but appeared to cause an increase in blood [Cl^-] (Fig. 2C). Low pH, 4.5, without added Al was associated with a further decline in blood [Na^+] (Fig. 2A) but no change in [Ca^{++}] or [Cl^-] (Fig. 2B,C). Aluminum at pH 4.5 exacerbated the effects by still further reducing [Na^+] and [Cl^-] (Fig. 2A,B) and elevating [Ca^{++}].

We hypothesize that $CaCO_3$ stores, probably in the shell, are mobilized to produce HCO_3^- for buffering the external acid. This could explain the elevated blood [Ca^{++}].

The laboratory experiment was useful *qualitatively* in separating the effects of acid and Al on blood ionic concentrations but the laboratory conditions did not *quantitatively* preserve the blood ionic composition seen in the field populations. The mussels appeared to be stressed by the laboratory conditions.

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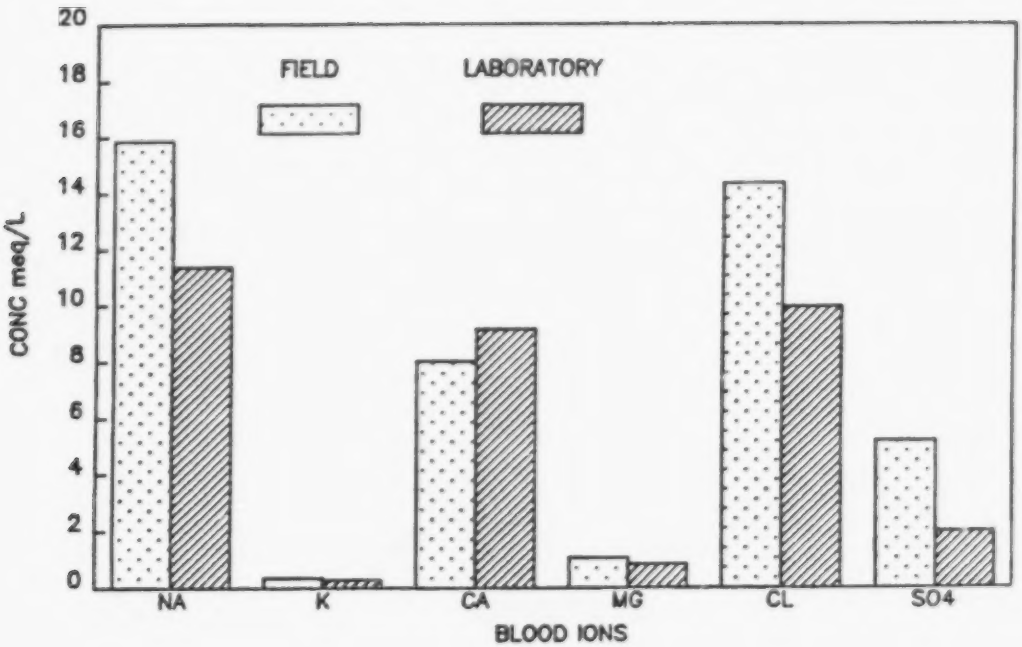

Fig. 1. Comparison of blood ionic concentrations from 30 mussels sampled in the field at ELA with those from 10 mussels sampled in the Freshwater Institute after 180 km transport and residence of one day in the Institute laboratory. For each ion, the field and laboratory concentrations are significantly different at the P=0.05 level as determined by one-way ANOVA.

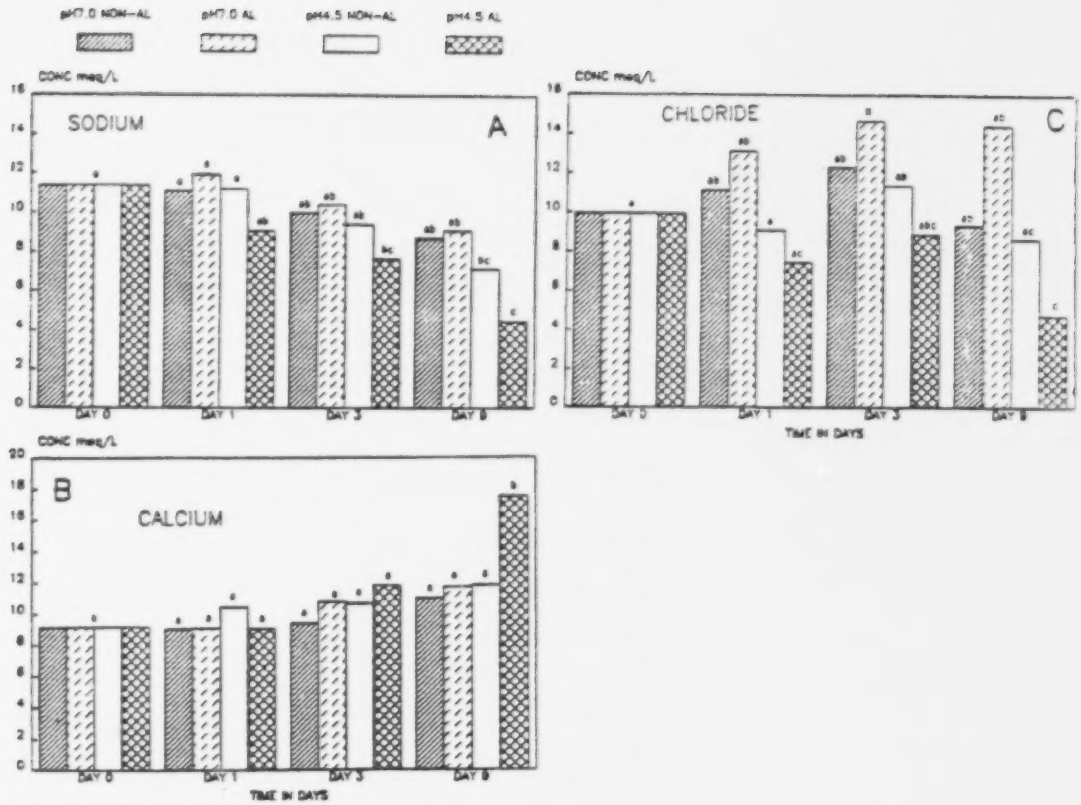

Fig. 2. Changes in blood concentrations of sodium (A), calcium (B) and chloride (C) in four acid - Al treatments over time. On day 0, blood from a single set of 10 mussels served as the baseline for all four treatments. For each ion, bars with the same letter are statistically not different at the P=0.05 level. Statistical difference is indicated by different letters.

ACCUMULATION OF ALKYLLEAD COMPOUNDS BY CAGED CLAMS

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ABSTRACT

Accumulation of chemicals by biota has been well known. In the aquatic ecosystems, direct uptake from water seems to be the main mechanism of bioconcentration. Studies on bioaccumulation have mainly been conducted in water systems without any regards to the sediment which is the ultimate sink of the aquatic system. Alkyllead compounds (R = Me, Et), as a result of anthropogenic input, have been found in sediment and in fish near the alkyllead manufacturers. These compounds are either physically bound by adsorption or chemically bound by complexation in sediment and are readily available to biota through leaching or exchange processes.

Environmental experiments using caged freshwater clams (*Elliptio complanata*) were conducted in areas of alkyllead contamination (e.g. St. Lawrence and St. Clair Rivers, Ontario). Over a three-month experiment period in the St. Lawrence River area, the highest accumulation was the tetraethyl-lead species (1.55 μg/g), followed by triethyl-lead (1.13 μg/g) and diethyl-lead (0.26 μg/g), calculated on whole clam, wet weight basis. The highest accumulation of alkyllead was found in the muscle and viscera tissues which accounted for ca. 65% of the total alkyl content.

Laboratory experiments in aquaria indicated that clams also accumulated alkyllead compounds from contaminated sediments. For example, with St. Clair River sediment, clams accumulated triethyllead, diethyl-lead and lead (II) at the rates of 1.96, 0.26, and 4.87 ng/g/d, respectively. No *in vivo* transformation or interconversion of the alkyllead species was evident.

RÉSUMÉ

L'accumulation de produits chimiques dans les biotes est un fait bien connu. Dans les écosystèmes aquatiques, l'absorption directe des composés dans l'eau semble le principal mécanisme de bioconcentration. Les études de bioaccumulation ont été surtout effectuées dans des systèmes aquatiques sans tenir compte des sédiments qui constituent le point d'élimination final pour les systèmes aquatiques. Des composés alkylés de plomb (R = Me, Et) d'orignie anthropique ont été trouvés dans les sédiments et dans la chair de poissons près d'usines produisant des composés alkylés de plomb. Ces composés sont liés physiquement par adsorption ou chimiquement par complexation aux sédiments et sont facilement accessibles à une reprise par les biotes par lessivage ou échange.

Des tests sur le terrain portant sur des bivalves d'eau douce cage (*Elliptio complanata*) ont été faits dans des secteurs contaminés par des composés alkylés de plomb (c.-à.-d. fleuve Saint-Laurent et rivière Sainte-Claire, Ontario). Sur une période de trois mois dans le secteur du fleuve Saint-Laurent, l'espèce chimique le plus accumulé chez les bivalves en poids frais detout l'organisme a été le plomb tétraéthylé (1.55 μg/g), suivie du plomb triéthylé (1.13 μg/g) et ensuite du plomb diéthylé (0.26 μg/g). La plus forte concentration de plomb alkylé a été observée

dans le muscle et les viscères; elle correspond à environ 65% de la teneur totale en alkyles.

Des expériences faites en aquarium au laboratoire ont indiqué que les bivalves concentraient également les composés alkylés de plomb contenus dans les sédiments contaminés. Par exemple, avec les sédiments de la rivière Sainte-Claire, les bivalves ont accumulé le plomb triéthylé, le plomb diéthylé et le plomb (II) aux taux de 1.96, 0.26 et 4.87 ng/g/d, respectivement. Aucune transformation *in vivo* ni interconversion des espèces alkylées de plomb n'était apparente.

EFFECT OF DIETARY DISODIUM ARSENATE HEPTAHYDRATE ON PROXIMATE DIET COMPONENT DIGESTIBILITY IN JUVENILE RAINBOW TROUT

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ABSTRACT

Juvenile rainbow trout were fed semi-purified diets containing graded levels of disodium arsenate, heptahydrate (DSA) to determine the effect of chronic exposure of dietary DSA on the digestibility of major dietary components by rainbow trout, and to confirm the signs of chronic toxicity to dietary DSA. Exposure of fish to 37 μg As/g diet as DSA did not significantly reduce either feed consumption or growth, but increased the feed:gain ratio, and reduced the apparent digestibility of the lipid and gross energy components of the diet from 94.4% to 88.1% to 85.7% respectively. Crude protein digestibility increased from 98.6%-99.1%. Exposure to 56 μg As/g diet as DSA caused significant reductions in feed intake and growth, and further increased the feed:gain ratio, as well as further reducing lipid and gross energy digestibility to 79.9% and 83.3% respectively.

Haematological assays demonstrated the existence of a microcytic anemia in the fish fed 56 μg As/g diet as DSA. Visible lesions of the gallbladder capsule were seen in 76% of the fish fed 37 μg As/g diet and 100% of fish fed 56 μg As/g diet as DSA. Histological examination of the affected gallbladders showed no changes in the mucosa, but extensive fibrosis, edema and mixed inflammatory cell infiltration in the submucosal tissues of the gallbladder well. [Study supported by NSERC and OMAF.]

RÉSUMÉ

Des truites arc-en-ciel juvéniles ont été nourries avec des rations alimentaires semi-purifiées et contenant des quantités mesurées d'arséniate disodique heptahydraté (ADH) afin de déterminer l'effet d'une exposition chronique par l'ADH dans les aliments sur la digestibilité des principales fractions alimentaires par la truites arc-en-ciel, et afin de confirmer les signes d'une toxicité chronique à l'ADH des aliments chez la truite.

L'exposition des poissons à 37 µg As/g dans la ration alimentaire sous forme d'ADH n'a pas réduit de façon significative la consommation d'aliments ou la croissance mais a accru le rapport aliments: gain et la digestibilité apparante de la fraction lipidique et des sources d'énergie brute de la ration alimentaire, de 94.4% à 88.1% et 85.7%, respectivement, tandis que la digestibilité des protéines crues est passée à 98.6% à 99.1%. L'exposition à 56 µg d'As/g d'aliments sous forme d'ADH a conduit à des réductions significatives de l'ingestion des aliments et de la croissance et a davantage accru le rapport aliments : gain en plus de réduir davantage la digestibilité des lipides et des sources d'energie brute à 79.9% et 83.3% respectivement.

Des tests hématologiques ont mis en évidence une anémie de type microcytique chez les poissons qui ont été nourris avec 56 µg As/g d'aliments sous forme d'ADH. Des lésions étaient apparantes sur la capsule de la vesticule biliaire chez 76% des poissons qui recevaient 37 µg As/g d'aliments et chez 100% des poissons qui recevaient 56 µg As/g d'aliments sous forme d'ADH. L'examen histologique des vésicules biliaires qui portaient des lésions n'a montré aucune modification des muqueuses, mais une fibrose extensive, l'oedème et une infiltration cellulaire inflammatoire mixte des tissus submuqueux du canal cholédoque. (Étude subventionnée par la *CRSNG* et l'OMAE.)

GLUTATHIONE-S-TRANSFERASE ISOENZYMES IN WHITE SUCKERS (*CATOSTOMUS COMMERSONI*) WITH POLLUTION ASSOCIATED SKIN AND HEPATIC NEOPLASMS

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ABSTRACT

Glutathione-S-transferase (TSGs) are a major group of cytosolic polypeptides that detoxify electrophilic xenobiotics by conjugating them with glutathione (GSH). We evaluated the levels and activities of GST by CDNB conjugation and polyacrylamide gel electrophoresis (PAGE) of cytosolic fractions of lip papilloma, surrounding lip, liver and liver neoplasms in white suckers from a polluted site in Lake Ontario. Lip tumors resembled normal lip tissue in their low content of GST activity and subunit bands by PAGE analysis. Levels of glutathione reductase (GR) and glutathione peroxidase (GPO) were elevated in papillomas. Hepatic GST activities were much greater in males than females. Hepatic GSTs from normal white suckers were purified by S-hexylglutathione affinity chromatography to 4 subunit bands by PAGE analysis. Affinity purified hepatic GSTs were used to prepare polyclonal antibodies to analyze tissue distribution of GSTs in suckers with normal or neoplastic liver lesions. Biliary neoplasms had reduced total GST levels, whereas hepatocellular neoplasms and preneoplastic lesions tended to have normal or elevated GST levels in comparison with surrounding liver. Renal tubules had very high levels of GST by immunohistochemical staining. These studies indicate that normal tissues of white suckers contain levels and isoenzyme profiles of GST similar to those with tissues of other species that are resistant to aflatoxin B_1 and benzo(a)pyrene. They suggest that immunohistochemical

staining for GST may be useful in detecting early hepatocellular and biliary neoplasms in fish.

RÉSUMÉ

Les glutathion-s-transférases (GST) constituent un groupe majeur de polypeptides cytosoliques qui détoxifient l'organisme de composés xénobiotiques électrophiles par conjugaison avec le glutathion (GSH). Nous avons évalué la concentration et l'activité de la GST par conjugaison (CDNB) et électrophorèse sur gel de polyacrylamide (PAGE) des fractions cytosoliques de papillomes des lèvres, du tissu environnant et du tissu du foie de néoplasmes du foie chez le meunier noir capturé dans un secteur pollué du lac Ontario. Les tumeurs des lèvres ressemblaient au tissu normal des lèvres par leur faible activité en GST et par les bandes correspondant aux sous-unités obtenues par électrophorèse. La glutathion-réductase (GR) et la glutathion-peroxydase (GPO) étaient concentration élevée dans les papillomes. L'activité des GST au niveau du foie était beaucoup plus élevée chez les mâles que chez les femelles. Les GST du foie meuniers noirs normaux étaient purifiées par chromatographie par affinité au S-hexylglutathion pour donner quatre bandes correspondant aux sous-unités obtenues par PAGE. Les GST hépatiques purifiées par affinité ont été utilisées pour l'obtention d'anticorps polyclonaux qui ont servi à étudier la distribution des GST dans les tissus de meuniers qui avaient un foie normal ou qui avaient des lésions néoplastiques. La concentration totale en GST était faible dans les néoplasmes biliaires alors qu'elle avait tendance à être normale ou élevée dans les néoplasmes hépatocellulaires et dans le tissus des lésions prénéoplastiques, par comparaison au tissu environnant du foie. La coloration immunohistochimique a montré que les tubules rénaux avaient une concentration très élevée de GST. Ces études montrent que le tissu normal du meunier noir contient des concentrations et présentent des profils isoenzmatiques de la GST semblables à ceux trouvés dans tissus d'autres espèces qui sont résistantes à l'aflatoxine B_1 et au benzo(a)pyrène. Les résultats semblent indiquer que la coloration immunohistochimique spécifique à la GST peut constituer un moyen commode de détection hâtive des néoplasmes hépatocellulaires et billiaires chez le poisson.

PHYTOPLANKTON DIFFERENTIAL SENSITIVITY STRESS: PREDICTING VULNERABILITY AND IDENTIFYING RESPONSE MECHANISMS

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ABSTRACT

Differential algal sensitivity to chronic pollutant stress explain how organic contaminants can disrupt initial community structure in natural assemblages by the displacement of sensitive dominants with more resistant species. Aspects of toxins tolerance and sensitivity were investigated in laboratory cultures of unicellular phytoplankton with the forest pesticide Fenitrothion as the chemical stressor. Characteristics of cell size, accumulation and depuration capacities and cellular lipid

levels were correlated with growth responses. Additional response patterns obtained suggest initial molecular effects are later manifested in changes at the population level. Fenitrothion-induced cell division inhibition and augmented macromolecule levels led to an increase in mean cell volumes and a consistent shift in cell size distributions within populations. This inquiry may aid in predicting which phytoplankton are at higher risks to pesticide contamination and elucidate those response mechanisms which initiated at the molecular level result in population and community alterations.

RÉSUMÉ

La sensibilité différentielle des algues à un stress chronique causé par des polluants montrent comment des contaminants organiques peuvent perturber la structure originale d'une communauté formée en assemlages naturels par le remplacement des dominants sensibles avec des espèces plus résistantes. Certains aspects de la tolérance et de la sensibilité aux toxines ont été études en laboratoire sur des cultures d'organismes phytoplanctoniques unicellulaires exposés au pesticide forestier Fenitrothion. Les dimensions cellulaires, la capacité d'accumulation et de dépuration ainsi que les concentrations cellulaires en lipides ont été mises en corrélation avec la croissance. D'autres courbes de réponse font penser que des effets initiaux au niveau moléculaire se répercutent ultérieurement au niveau démographique. L'inhibition de la division cellulaire induite par le Fenitrothion et l'augmentation de la concentration des macromolécules ont conduit à une augmentation moyenne du volume des cellules et à un déplacement correspondant des distributions de taille des cellules dans les populations. La présente étude peut aider à prévoir quels organismes phytoplanctoniques sont les plus ménacés par une contamination aux pesticides, et peut aider à comprendre comment les mécanismes de réaction au niveau moléculaire se traduisent par des changements de population et de composition des communautés.

INFLUENCE OF CARCINOGENS ON ATTACHED MONOLAYERS CULTURES OF HEPATOCYTES FROM RAINBOW TROUT

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ABSTRACT

Hepatocarcinogens, including aflatoxin B_1 (AFB_1) and 2-acetylaminofluorene (2-AFF) elicit several toxic responses in mammalian liver cells, namely necrosis, unscheduled DNA synthesis (UDS) or inhibition of replicative DNA synthesis (RDS). These responses are assayed routinely in primary attached monolayer cultures prepared from collagenase dissociated livers. We developed a similar method for trout hepatocytes, which were attached to collagen-coated plastic dishes at 10°C in L15 medium containing 5-10% trout serum. AFB_1 (1-10 nM) and acetaminophen (16 mM) caused release of lactate dehydrogenase from trout hepatocytes, similar to necrogenic influences of these agents on mammalian hepatocytes. However, UDS or RDS responses could not be detected in trout hepatocytes by the use of ^3H-labelled thymidine

(TdR). By comparison 2-AFF and AFB[1] elicited strong UDS and inhibited RDS responses to epidermal growth factor in monolayer cultures of rat hepatocytes. Available ^{3}H-TdR was totally lost within 2 h in medium with trout hepatocytes, but remained present for up to 72 h in medium with rat hepatocytes. Culture medium containing ^{3}H-TdR obtained from 0, 3, 24 and 48 h trout hepatocyte cultures was fractionated by sephacryl S-200 gel filtration. Within 3 h, most ^{3}H activity was present in a new peak that eluted in advance of ^{3}H-TdR or unlabelled TdR. These studies indicate that attached monolayer cultures of trout hepatocytes are sensitive to mammalian hepatocarcinogens but do not exhibit UDS or RDS responses with ^{3}H-TdR because they rapidly metabolize the tracer to a non-utilizable product.

RÉSUMÉ

Les hépatocarcinogènes, notamment l'aflatoxine B_1 (AFB_1) et le 2-acétylaminofluorène (2-AFF) donnent lieu à plusieurs réactions de toxicité dans les cellules du foie des mammifères, notamment des nécroses, la synthèse non programmée de DNA (SDNA) ou l'inhibition de la réplication de DNA (RDNA). Ces réactions sont measurées de façon courante en culture monocouche primaire fixée préparée à partir de foies traités à la collagénase. Nous avons mis au point une méthode apparentée pour les hépatocytes chez la truite qui sont fixés à des vases en plastique enduits de collagène, à 10°C dans le milieu L15 à 5-10% de sérum de truite. L'AFB[1] (1-10 nM) et l'acétaminophène (16 mM) ont provoqué le dégagement de lactate déshydrogénase par les hépatocytes de la truite, ce qui est semblable aux effets nécrotiques de ces agents sur les hépatocytes de mammifères. Cependant, l'effet sur la SDNA ou la RDNA n'a pas pu être détecté avec l'emploi de ^{3}H-thymidine sur les hépatocytes de la truites. Par comparaison, le 2-AFF et l'AFB[1] ont suscité une forte SDNA et inhibé la réaction de RDNA au facteur de croissance épidermique dans des cultures en couche monocellulaire d'hépatocytes de rat. Avec les hépatocytes de truite, la ^{3}H-thymidine a été totalement perdue dans le milieu en 2h, mais elle est restée présente jusqu'à 72 h avec les hépatocytes de rat. Le milieu de culture contenant de la ^{3}H-thymidine obtenue de cultures d'hépatocytes de truite après 0, 3, 24 et 48 h, a été fractionné par filtration sur gel séphacryl S-200. En 3 h, la majeure partie de l'activité ^{3}H a été concentrée dans la fraction correspondant à un niveau nouveau pic élué avant la ^{3}H-thymidine ou la thymidine non marquée indiquent que les cultures en couche monocellulaire d'hépatocytes de truite sont sensibles aux hépatocarcinogènes chez les mammifères, mais n'ont pas les réactions de SDNA ou de RDNA en présence de ^{3}H-thymidine parce qu'ils métabolisent rapidement le traceur en un produit non utilisable.

AQUATIC FATE AND EFFECTS OF 2,3,4,6-TETRACHLOROPHENOL

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ABSTRACT

The environmental fate and toxicity of the common wood preservative 2,3,4,6-tetrachlorophenol (TTCP), was studied in a lentic freshwater ecosystem using aquatic enclosures, isolating a 125 m^3 column of water.

Treatments yielding initial concentrations of 0.2 and 2.0 mg/L were monitored with respect to persistence in the water and sediment, and potential for adsorption onto the plastic enclosure wall. Negligible residues were found in sediment and enclosure walls. Degradation in water was rapid, yielding less than 1% of the initial concentration after 6 wk.

A significant decrease was observed in primary productivity at the high treatment concentration as determined by decrease in chlorophyll *a* levels. Zooplankton populations were also affected at the high TTCP treatment. A significant decrease in numbers was observed within the first wk after treatment. Cladocera were in general affected more than copepods, while rotifers exhibited a slight increase in numbers coinciding with the decrease in other groups. No significant change was observed in either the zooplankton or phytoplankton populations at the low treatment concentration.

RÉSUMÉ

Ce qu'il advient dans le milieu de l'agent de traitement du bois à l'usage répandu, le 2,3,4,6 tétrachlorophénol (TTCP) ainsi que sa toxicité ont été étudiés dans un écosystème lentique d'eau douce au moyen d'enceintes aquatiques qui se trouvaient à isoler un volume de 125 m^3 d'eau.

La persistance dans l'eau et les sédiments ainsi que le potentiel d'adsorption sur les parois en plastique de l'enceinte ont été étudiés après application du produit en concentrations de 0.2 et 2.0 mg/L. Des résidus en quantité négligeable ont été trouvés dans sédiments et sur les parois de l'enceinte. La décomposition du produit dans l'eau était rapide avec seulement moins de 1% de la concentration initiale après 6 semaines.

La mesure de la concentration en chlorophylle *a* a montré qu'il s'est s'est produit une importante diminution de productivité primaire à la plus forte concentration. Les population de zooplancton ont également souffert du produit à la concentration la plus élevée. Une diminution significative en nombre a été observée au bout d'une semaine après le traitement. Les cladocères ont généralement été plus affectés que les copécodes tandis que les rotifères se sont légèrement accrus en nombre, en même temps que d'autres groupes perdaient de l'importance. Aucun changement significatif n'a été observé dans les populations de zooplancton ou de phytoplancton à la concentration la plus faible.

CHEMICAL EVALUATION SEARCH AND RETRIEVAL SYSTEM (CESARS)

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ABSTRACT

CESARS (Chemical Evaluation Search and Retrieval System) is an environmental toxicological database developed by the Michigan Department of Natural Resources. It presently contains profiles for approximately 200 substances of particular significance to the Great Lakes. The information is divided into the following topic areas:

- Physical & Chemical Properties
- Regulations & Guidelines
- Manufacture
- Acute Toxicity
  - Terrestrial Life
  - Humans
  - Aquatic Life
- Chronic Toxicity
  - Terrestrial Life
  - Humans
  - Aquatic Life
- Phytotoxicity
- Carcinogenicity
- Mutagenicity
- Reproductive & Developmental Effects
- Other Adverse Effects
- Pharmacokinetics
- Bioaccumulation/Bioconcentration
- Transport Processes
- Environmental Fate Processes
- Transformation Processes
- Analysis & Treatment

Under each topic, studies are summarized outlining results and methodology, to assist in the assessment of the environmental toxicology and fate of a substance. New profiles will include summary fields that will include an evaluation of the information presented.

RÉSUMÉ

CESARS (Chemical Evaluation Search and Retrieval System) est une base de données toxicologiques du milieu développée par le *Michigan Department of Natural Resources*. Ce système offre le profil d'environ 200 substances d'importance particulière dans la région des Grands Lacs. Les renseignements qui y sont contenus sont répartis selon les domaines suivants:

- Propriétés physiques et chimiques
- Règlements et lignes directrices
- Fabrication
- Toxicité aiguë
  - Vie terrestre
  - Humains
  - Vie aquatique
- Toxicité chronique
  - Vie terrestre
- Phytotoxicité
- Cancérogénicité
- Mutagénicite
- Effets sur la reproduction et le développement
- Autres effets nocifs
- Pharmococinétique
- Bioaccumulation-bioconcentration
- Mécanisme de transport
- Mécanisms liés au destin produit dans le milieu

Humains
Vie aquatique

Mécanismes de transformation

Sous chaque en-tête, on présente un résumé de études avec une description rapide des résultats et de la méthodologie pour aider à l'évaluation de la toxicologie des substances considérés dans le milieu et de leurs sort. Les nouveaux profils comprendront des domaines en résumé qui porteront notamment sur l'évaluation de l'information présentée.

FACTORS THAT CAN INFLUENCE DIETARY ABSORPTION EFFICIENCY OF CHEMICALS BY FISHES

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ABSTRACT

It has been suggested that chemicals with molecular weights greater than 600 are poorly absorbed by fish. This observation could have significant implications on contaminant monitoring programs that use fish as an indicator species, and application of contaminant dynamics models which includes a variable to represent chemical uptake efficiency. Rainbow trout (*Salmo gairdneri*) were exposed to 17 organic compounds with molecular weights of 266-627 to examine the influence of molecular weight and molecular volume on dietary absorption efficiency. Molecular volume can be an important variable because the relationship between weight and volume among chemicals can differ by several fold depending on elemental composition and structural arrangement of the elements. The results of this and other studies indicated absorption of chemicals by salmonids were poor as molecular weights exceeded 600, but some chemicals of lower molecular weight were also poorly absorbed. A slightly better trend was suggested with molecular volume where chemicals with volumes over 0.30 nm^3 were poorly absorbed, although neither index provided a consistent relationship that could be consistently applied to all chemicals.

RÉSUMÉ

Il a été avancé que les produits chimiques dont le poids moléculaire est supérieur à 600 sont mal absorbés par le poisson. Cette observation pourrait avoir de profondes conséquences sur les programmes de surveillance des contaminants qui utilisent les poissons comme indicateurs ainsi que sur l'application de modèles de la dynamique des contaminants qui ont une variable correspondant à l'efficacité de l'absorption de produits chimiques. Des sujets de truite arc-en-ciel (*Salmo gairdneri*) ont été exposés à 17 composés organiques dont le PM varait entre 266 et 627, cela afin d'examiner l'influence du PM et du volume moléculaire sur l'efficacité de l'absorption avec les aliments. Le volume moléculaire peut constituer une variable importante car le rapport entre le PM et le volume des molécules peut varier de ordres de grandeur d'un composé à l'autres selon la composition élémentaire et la géométrie des molécules. Les résultats obtenus avec cette étude et d'autres indiquent que l'absorption

des produits chimiques par les salmonidés est mauvaise quand le PM dépasse 600, mais que certains produits chimiques de PM inférieur sont également mal absorbés. Les résultats ont été un peu meilleurs avec le volume moléculaire car les produits chimiques dont des volumes dépassent 0.3 nm^3 étaient mal absorbés; cependant, aucun des deux indices constitués n'a donné de rapport qui pouvait être appliqué de façon uniforme à tous les produits chimiques.

THE ACUTE LETHALITY OF POTASSIUM CYANATE AND POTASSIUM THIOCYANATE TO RAINBOW TROUT AS INFLUENCED BY WATER HARDNESS AND PH

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ABSTRACT

The Mining, Minerals and Metallurgical Processes Division, Industrial Programs Branch, Conservation and Protection, Ottawa, is in the process of assessing wastewater treatment technologies for the gold mining industry. Many of the treatment technologies involve the oxidation of cyanides to cyanates. Also, some effluents contain thiocyanate compounds which are more resistant to certain destruction methods. Existing data on the aquatic toxicity of cyanates and thiocyanates was both scarce and somewhat inconsistent. Therefore, this study was undertaken to assess the acute lethality of both a cyanate compound and a thiocyanate compound and to determine how that toxicity was affected by changes in solution hardness or pH.

Test compounds chosen for study were potassium cyanate (KCNO) and potassium thiocyanate (KCNS). Tests were 96 h static acute lethal toxicity tests using fingerling rainbow trout as the test organism. Tests were conducted at 15°C, and nominal concentrations of test materials were confirmed by chemical analyses. Both compounds were tested at pH values of 5, 7 and 8.5 (water hardness held constant). The toxicity of KCNO was significantly higher at the lowest pH tested, and while there was an indication that the lower pH also increased the toxicity of KCNS, the difference was not as clear cut (Table 1a).

Both compounds were also tested at nominal hardness of 20, 50, 100 and 250 mg/L (as $CaCO_3$). Water hardness did not affect the toxicity of KCNO, while increasing water hardness lead to increased toxicity of KCNS. Results of the toxicity tests are presented in Table 1b.

From the data, it would appear that the presence of thiocyanate in gold mill effluents should not be a primary source of acute toxicity. However, the formation of cyanate by the oxidation of cyanide can result in acutely lethal levels of cyanate in treated gold mill effluents.

RÉSUMÉ

La division des opérations minières et métallurgiques, direction des programmes industriels, conservation et protection, Ottawa, a commencé l'examen des techniques de traitement des eaux usées produits par l'extraction de l'or. Bon nombre des traitements comprennent une étape d'oxydation des cyanures en cyanates. En outre, certains effluents contiennent des composés du thiocyanate qui résistent davantage à certaines méthodes de décomposition. Les données connues sur la toxicité des cyanates et des thiocyanates dans l'eau sont rares et peu uniformes. C'est ce qui a donné lieu à la présente étude qui porte sur l'évaluation de la létalité aiguë d'un composé de cyanate et d'un composé de thiocyanate, et sur la façon dont la toxicité est modifiée par des changements du dureté ou de pH des solutions.

Le cyanate de potassium (KCNO) et le thiocyanate de potassium (KCNS) ont servi aux essais. Il s'agissait de tests statiques de létalité aiguë (96 h) qui utilisaient les alevins (fingerling) de truite arc-en-ciel comme organismes cibles. Les tests ont été faits à 15° C et les concentrations de référence des produits ont été vérifiées par dosage. Les deux composés ont été testés à pH 5, 7, et 8.5 (dureté de l'eau constante). La toxicité du KCNO était significativement supérieure au plus bas pH testé; bien qu'il semble que le plus bas pH ait également accru la toxicité du KCNS, la différence n'était cependant pas aussi évidente que dans l'autre cas (tableau 1a).

Les deux composés ont également été testés dans l'eau ayant une dureté de référance de 20, 50, 100 et 250 mg/L (sous forme de $CaCO_3$). La dureté de l'eau n'a pas influencé la toxicité du KCNO alors qu'un accroissement de dureté de l'eau a conduit à un accroissement de la toxicité du KCNS. Les résultats de ces tests de toxicité sont présentés au tableau 1b. D'après les résultats, il semble que la présence de thiocyanate dans les effluents des extracteurs d'or ne doit pas constituer une source primairede toxicité aiguë. Cependant, la formation de cyanate par oxydation du cyanure peut conduire à des concentrations de létalité aiguë de cyanate dans les effluents traités des extracteurs.

Table 1a. Toxicity of cyanate and thiocyanate related to pH.

Nominal pH	Toxicity of cyanate (96 h LC50 in mg/L)	Toxicity of thiocyanate (96 h LC50 in mg/L)
5	12.5 (10-14.9)	144 (106-180)
7	38.8 (32.3-43.9)	233 (154-311)
8.5	41.1 (31.5-50.7)	203 (100-413)

Table 1b. Toxicity of cyanate and thiocyanate related to hardness.

Nominal hardness mg/L	Toxicity of cyanate (96 h LC50 in mg/L)	Toxicity of thiocyanate (96 h LC50 in mg/L)
20	30.7 (20.2-40.1)	238 (160-316)
50	26.1 (22.5-30)	227 (175-278)
100	24.3 (16.8-31.8)	192 (121-263)
250	24.3 (16.8-31.8)	132 (93-170)

CANADIAN WATER QUALITY GUIDELINES AND THEIR USE IN WATER QUALITY MANAGEMENT

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ABSTRACT

The first edition of the Canadian Water Quality Guidelines was published by the Canadian Council of Resources and Environment Ministers in May 1987. The guidelines were prepared by the CCREM Task Force on Water Quality Guidelines, composed of federal, provincial and territorial government scientists. The Canadian Water Quality Guidelines provide information about the effects of water quality on water resources in Canada. The water resources covered in the Guidelines document include raw water for drinking water sources, recreational water and aesthetic considerations, freshwater aquatic life, agricultural water supplies including irrigation and livestock water, and industrial water supplies.

As a starting point, the CCREM Task Force examined water quality guidelines from all sources to see whether they were applicable to Canadian environmental conditions; if they were, then they were adopted as Canadian guidelines. If they were not applicable, but suitable information was available, they were modified to reflect Canadian conditions. In some cases where guidelines were lacking, but reliable scientific information was available, new water quality guidelines were developed. Where information was lacking to develop new guidelines, research needs were communicated to CCREM. The Canadian Water Quality Guidelines will be updated as new relevant information becomes available and where the need arises to revise existing guidelines and develop new ones.

Water quality guidelines form one component of an effective and efficient strategy for managing Canada's water resources. When used in the development of water quality objectives for specific water basins, sufficient site information must be generated to modify the guidelines to reflect local conditions. Water quality guidelines cannot be universally applied to all water resources throughout Canada. Watershed assessments, including information on the influence of hydrological, chemical, physical, biological and socio-economic factors, must be conducted so that reliable water quality objectives for specific sites can be developed and implemented.

RÉSUMÉ

Les recommendations pour la qualité des eaux au Canada, primière édition, ont été publiées par le Conseil canadien des ministres des Ressources et de l'environnement en mai 1987. Les lignes directrices ont été préparées par le groupe de travail sur les recommendations pour la qualité de l'eau du CCMRE, qui réunissait les chercheurs de fédéral, des provinces et des territoires. Les recommendations présentent des renseignements sur les effets de la qualité de l'eau sur les ressources en eau au Canada. Les ressources considérées sont l'eau brute comme source d'eau potable, les eaux servant à la récréation et à des fins esthétiques, l'eau douce et la protection des formes de vie aquatique, les réserves d'eau à des fins agricoles, notamment l'eau d'irrigation et l'eau pour le bétail, ainsi que les réserves d'eau industrielle.

Le groupe de travail du CCMRE a commencé par examiner les recommandations concernant la qualité de l'eau de toutes les sources pour voir si elles étaient applicables aux conditions canadiennes; lorsqu'elles l'étaient, ces normes étaient adaptées comme normes canadiennes. Lorsqu'elles ne l'étaient pas, mais que des renseignements utiles pouvaient en être tirés, ces renseignements étaient adaptés aux conditions canadiennes. Dans certains cas où il n'existait pas de recommendations, mais que des résultats scientifiques faibles étaient disponibles, de nouvelles recommendations ont été rédigées. Lorsqu'il n'y avait pas de matériel suffisant pour rédiger de nouvelles recommendations, le conseil des ministres s'était alors informé des besoins sur le plan de la recherche. Les recommendations pour la qualité de l'eau au Canada seront tenues à jour selon les progrès de la recherche et quand se fera sentir le besoin de reviser les lignes directrices en place et d'en créer de nouvelles.

Les recommendations pour la qualité de l'eau constituent un élément d'une stratégie efficace et méthodique de la gestion des ressources en eau du Canada. Lorsqu'elles sont appliquées à la préparation d'objectifs de qualité de l'eau des bassins déterminés, il faut obtenir tous les renseignements particuliers nécessaires pour modifier les recommendations afin de tenir compte des conditions locales. Les recommendations pour la qualité de l'eau ne peuvent pas être appliquées universellement à toutes les ressources en eau du pays. Il est nécessaire de procéder à des évaluations des bassins versant, et de recueillir notamment des renseignements sur l'influence de facteurs hydrologiques, chimiques, physiques biologiques et socio-économiques pour pouvoir préparer et appliquer des objectifs faibles de qualité de l'eau pour des secteurs donnés.

ASSESSING THE PRESENCE OF MICROENCAPSULATED PESTICIDES IN BIOLOGICAL SYSTEMS: A FIRST STEP

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ABSTRACT

The use of microencapsulated pesticides is proposed to overcome the problems of short residual activity of many modern pesticides. Microencapsulation consists of surrounding the active ingredient with a protective layer of inert material, usually a cross-linked polymer. The pesticide is then either ingested by the target organism or is slowly released in its environment. The significance of these formulations in biological systems needs to be addressed. In this study we have (1) evaluated staining and Scanning Electron Microscopy (SEM) as visual techniques to detect and quantify microcapsules in selected substrates; and (2) initiated the development of a protocol to integrate these procedures in the overall scheme of environmental impact assessment.

Five formulations of insecticides were studied: Penncap-M (methyl-parathion), Dyfonate (fonofos), Penncapthrin (permethrin) and Altosid, formulations SR-10 and SR-20 (methoprene). Aquatic invertebrates, mosquito larvae (*Culex pipens*) and *Daphnia magna* were exposed for 24 h to 1 ml/L of formulation. Two stains were studies: Methylene Blue, a water soluble stain and Sudan IV a lipophilic stain. For SEM, specimens were sputter coated with 60:40 gold:palladium at a thickness of 200-300 A. Analysis was conducted with a Hitachi S-570 scanning electron microscope. Three sample preparation procedures were evaluated; smears, isolation of the gut and microtomy.

With Methylene Blue, neither of the Penncap-M, Dyfonate or ME Permethrin stained; both methoprene formulations stained dark blue; all organic material surrounding the microcapsules was also stained. The absence of selectivity made microcapsule recognition impossible.

With Sudan IV, the microcapsules of Penncap-M, Dyfonate and Penncapthrin brightly stained; the surrounding material did not stain; none of the methoprene formulations stained.

Of the various sample preparation methods studied we found that (1) smears was easy, at the cost of a loss of information in terms of microcapsules location, and quantification is possible, (2) isolation of the gut was an interesting method, when applicable, however, preparation can be tedious, and quantification would be difficult, and (3) microtomy was a time consuming method, but there is potential for studies dealing with the rates of ingestion and excretion of the microcapsules i.e. quantification and location possible.

The results obtained in this study constitute a preliminary basis for integration of ME formulations within the overall scheme of environmental impact assessment. The procedures presented still need to be refined and in some cases the problem has to be approached from a different angle i.e. absence of selectivity with methoprene. These procedures could be integrated into toxicological and environmental studies.

RÉSUMÉ

Il est proposé d'utiliser des pesticides microencapsulés pour surmonter les problèmes liés à la courte activité résiduelle de bon nombre des pesticides modernes. La microencapsulation consiste à entourer l'ingrédient actif d'une couche protectrice d'un matériel inerte, le plus souvent une polymère à réticulation croisée. Le pesticide est ensuite ingéré par l'organisme cible ou bien est libéré lentement dans son milieu. Il est nécessaire d'étudier l'effet de ces formulations sur les systèmes biologiques. Dans la présente étude, nous avons 1) évalué la coloration et la microscopie électronique à balayage comme techniques visuelles de détection et de quantification des microcapsules dans certains substrats et 2) nous avons commencé la rédaction d'un protocole visant à intégrer ces méthodes dans un plan d'ensemble d'évaluation des répercussions sur le milieu.

Cinq formulations d'insecticides ont été étudiées : le penncap-M (méthyl-parathion), le dyfonate (fonofos), le penncapthrin (perméthrin) et l'Altosid, formulations SR-10 et SR-20 (méthoprène). Des invertébrés aquatiques, des larves de moustiques (*Culex pipens*) et *Daphnia magna* ont été exposés pendant 24h à 1 ml/L des formulations. Deux colorations ont été étudiées. Le bleu de méthylène, un colorant soluble dans l'eau et le Sudan IV, un colorant lipophile. Les spécimens passés au microscope électronique étaient recouverts d'une couche pulvérisée d'un mélange or-palladium dans les proportions 60-40 et épaisse de 200-300 A^0. Le microscope à balayage était un Hitachi S-570. Trois méthodes de préparation des échantillons ont été évaluées : les frottis, l'isolement du système digestif et la microtomie.

Le Penncap-M, le Dyfonate et le ME perméthrin n'ont pas été colorés par le bleu de méthylène; les deux formulations de méthoprène ont été colorées en bleu foncé; tout le matériel organique qui entourait les microcapsules a également été coloré. L'absence de sélectivité a rendu impossible la détection des microcapsules.

Les microcapsules de Penncap-M, de Dyfonate et de Penncapthrin ont été colorées brillamment par le Sudan IV; le matériel environnant n'a pas été coloré; aucune des formulations du méthoprène n'a été colorée.

Après l'examen des différentes méthodes de préparation choisie, nous avons constaté que 1) les frottis se faisaient bien, mais au prix d'une perte d'information (emplacement des microcapsules) et la quantification est possible, 2) l'isolement du système digestif est une méthode intéressante quand elle est applicable, seulement la préparation risque d'être fastidieuse et la quantification dificile, 3) la microtomie est une méthode qui prend beaucoup de temps, mais elle offre du potentiel avec les études qui traiteraient des taux d'indigestion et d'excrétion de microcapsules, c'est-à-dire qu'elle rend la quantification et la localisation possibles.

Les résultats obtenus ici constituent une base préliminaire qui devrait aider à l'intégration des formulations de ME dans le plan global d'évaluation des incidences écologiques. Les méthodes décrites doivent être raffinées, et dans certains cas le problème doit être abordé de façon différente : on pense ici au manque de selectivité avec le méthoprène. Ces méthodes pourraient être intégrées dans des études toxicologiques et écologiques.

REGRESSION AND DEVELOPMENT OF SKIN PAPILLOMAS AFFECTING WHITE SUCKERS (*CATOSTOMUS COMMERSONI*) FROM POLLUTED AREAS IN LAKE ONTARIO

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ABSTRACT

Epidermal papillomas are frequently found on white suckers (*Catostomus commersoni*), from various regions of the Great Lakes of North America, especially in industrially polluted sites. White suckers with morphologically distinct but histologically similar types of papillomas on their lips and body were collected from a Lake Ontario spawning stream. The diameters of focal soft skin plaques, focal hard skin papillomas and focal lip tumors were monitored on individual fish before and after a 12-wk observation period in "clean" laboratory tanks. Many papillomas disappeared completely, while others persisted. Lip regressed less frequently (22%), compared to skin plaques (79%) and skin papillomas (64%). Papillomas which persisted had only slightly smaller diameters after 12 wk. New tumors developed on 23 and 60% of the survivors in two separate groups. The development of new tumors in laboratory conditions suggests that these papillomas may be promoted by influences unrelated to environmental pollution.

RÉSUMÉ

Des papillomes épidermiques sont fréquemment trouvés sur les meuniers noirs (*Catostomus commersoni*) provenant de différentes régions des Grands lacs, notamment des secteurs atteints par la pollution industrielle. Dans un cours d'eau qui sert à la fraie et qui se déverse dans le lac Ontario, on a capturé des meuniers noirs porteurs de papillomes morphologiquement distincts, mais histologiquement semblables, sur les lèvres et le corps. On a mesuré régulièrement le diamètre des plaques d'épiderme mou en foyer, de papillomes de l'épiderme indurés en foyer et de tumeurs en foyer sur les lèvres de sujets suivi un à un avant et après une période d'observation de 12 semaines dans des bassins "d'eau propre" au laboratoire. Beaucoup des papillomes sont entièrement disparus alors que d'autres ont persisté. Ceux de la lèvre ont régressé moins fréquemment (22%) que les plaques épidermiques (79%) et les papillomes épidermiques (64%). Les papillomes qui ont persisté avaient atteint un diamètre à peine inférieur après 12 semaines. De nouvelles tumeurs se sont développées chez 23 et 60% des survivants de deux groupes distincts. L'apparation de nouvelles tumeurs dans des conditions de laboratoire semble indiquer que ces papillomes peuvent être activés par des facteurs qui sont sans rapport avec la pollution.

PERSISTENCE AND DISSIPATION OF FORESTRY HERBICIDES IN A NORTHERN ONTARIO LAKE

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ABSTRACT

A field study was conducted in enclosures located in a typical bog lake in a sandy soil area near Matheson in northeastern Ontario. Three groups of 6 enclosures each were constructed and set up in the lake. Three enclosures each were treated with triclopyr (butoxyethanol ester), with 2,4-D (isooctyl ester) and with hexazinone solution at rates equivalent to 0.3 and 3.0; 1.0 and 2.5; 0.4 and 4.0 kg/ha respectively. Water, sediment, enclosure wall samples were analyzed for residues and temperature and oxygen levels were measured.

A significant dose-dependent change in oxygen concentration was only seen in the hexazinone treated enclosures. Rates of dissipation of 2,4-D were similar at both concentrations and, within 15 d less than 5% remained in the water. Up to 25% of the 2,4-D adsorbed to the sides of the corrals. Triclopyr concentration in water was below 5% from day 15 and could not be detected from day 42 onwards. The amount of pesticide absorbed to the sides of the enclosures was lower and appeared to dissipate more rapidly than was the case with 2,4-D. At the lower application rate, hexazinone was undetectable 21 d and at the higher rate 42 d after application. Hexazinone dissipated more rapidly than 2,4-D and was not adsorbed to sediments.

RÉSUMÉ

Une étude a été dans des enceintes disposées dans un lac-tourbière typique constitué dans un sol sablonneux près de Matheson dans le nord-est de l'Ontario. Trois groupes de six enceintes chacun ont été mis en place. Trois enceintes de chaque groupe ont été traitées au triclopyr (ester de butoxyéthanol), au 2,4-D (d'ester isooctyle) et avec une solution d'hexazinone à des doses équivalant à 0.3 et 3.0, 1.0 et 2.5, 0.4 et 4.0 kg/ha, respectivement. Les résidus ont été dosés dans l'eau, les sédiments et des fragments des parois des enceintes, et la température et la teneur en oxygène ont été mesurées.

Une variation significative de la teneur en oxygène reliée à la dose a été observée seulement dans les enceintes traitées à l'hexazinone. Les taux de dissipation du 2,4-D étaient les mêmes aux deux concentrations et, en moins de 15 jours, moins de 5% du produit restait dans l'eau. Jusqu'à 25% du 2,4-D a été adsorbé sur les parois des enceintes. La concentration du triclopyr dans l'eau était inférieure à 5% au jour 15 et ne pouvait plus être détectée après le jour 42. La quantité de pesticide adsorbée par les parois des enceintes était inférieure et semblait se dissiper plus rapidement que ce ne fut le cas avec le 2,4-D. L'hexazinone à la faible concentration n'était plus détectable après 21 jours et ne l'était plus à la concentration élevée après 42 jours. L'hexazinone s'est dissipé plus rapidement que le 2,4-D et n'a pas été adsorbé par les sédiments ou les parois des enceintes.

HORMONAL REGULATION OF GLUCONEOGENESIS IN BROOK TROUT (*SALVELINUS FONTINALIS*) MAINTAINED IN LOW ENVIRONMENTAL PH

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ABSTRACT

Increased cortisol secretion and plasma glucose concentrations are normal responses of teleosts to many types of stress stimuli, including acid exposure. The functions ascribed to cortisol in fish are iono-osmoregulation and carbohydrate metabolism. Yet, our previous experiment showed that although injection of cortisol acetate significantly raised the levels of circulating cortisol in brook trout, the plasma concentration of glucose remained at control values, suggesting that cortisol alone could not affect blood glucose levels. The aim of this study is to prove the hypothesis that high blood glucose levels observed in acid-stressed brook trout are brought on by the suppression of insulin or insulin-like factors and the enhancement of cortisol secretion. Yearling brook trout were maintained at pH 4.5 (sulphuric acid) and given 4 injections of either vehicle, metyrapone or insulin over a period of 8 d. In the untreated (injection vehicle) acid-exposed fish, plasma levels of glucose and amino acids were increased, but plasma osmolarity was decreased. In the metyrapone injected trout, cortisol secretion was suppressed. Plasma levels of amino acids returned to normal, but glucose levels remained high. When insulin was administered to the acid-treated trout, both plasma concentrations of glucose and amino acids became significantly lower than those of control fish. The results show that the extra energy required by the acid-treated trout for iono-osmoregulation comes from the protein reserves. Cortisol is needed for converting protein into amino acids, but it is the suppression of insulin or insulin-like factors that directly causes plasma glucose levels to rise.

RÉSUMÉ

L'augmentation de la sécrétion de cortisol et celle de la concentration de glucose plamisque constituent des réactions normales des téléostéens à de nombreux stimuli différents, notamment l'exposition à des pH acides. Les fonction rattachées au cortisol chez les poissons comprennent l'iono-osmorégulation et le métabolisme des hydrates de carbone. Et pourtant, lors de notre expérience antérieure, nous avions observé que l'injection d'acétate de cortisol faisait augmenter de façon significative la concentration de cortisol circulant chez l'omble de fontaine, mais que la concentration en glucose du plasma restait au niveau de référence ce qui semblait indiquer que le cortisol seul ne pouvait pas agir sur la teneur sanguine en glucose. L'objet de la présente étude est de confirmir l'hypothèse à l'effet que les concentrations sanguines élevées de glucose qui sont observées chez l'omble de fontaine stressées par un pH acide résultent de la suppression de l'insuline ou de facteurs apparentés à l'insuline et de la sécrétion accrue de cortisol. Des ombles de fontaine d'un an ont été gardés à pH 4.5 (acide sulfurique) et on reçu quatre injections d'un vecteur, de métyrapone ou d'insuline sur une période de 8 jours. Chez les sujets non traités (injection du vecteur), la teneur en glucose et en acides aminés dans le plasma se sont accrues, mais l'osmolarité du plasma a diminué. Chez les ombles qui ont reçu des injections de métyrapone, la sécretion de cortisol a été supprimée. La teneur en

acides aminés du plasma est revenue à la normale, mais la teneur en glucose est restée élevée. Quand de l'insuline a été administrée aux ombles traités à l'acide, la teneur en glucose et en acides aminés du plasma s'est abaissée de façon significative par rapport à la teneur chez les groupes témoins. Les résultats montrent que l'énergie supplémentaire qui est nécessaire à l'iono-osmorégulation chez les ombles traités à l'acide est puisée dans les réserves protéiniques. Le cortisol est nécessaire pour la conversion des protéines en acides aminés, mais c'est la suppression de l'insuline et des facteurs apparentés à l'insuline qui provoque directement l'augmentation de la teneur en glucose du plasma.

INTRODUCTION

Increased cortisol secretion and plasma glucose concentrations are the usual response of teleosts to many types of stressful stimuli, including acid exposure (Brown et al. 1984; Tam et al. 1987a). The functions ascribed to cortisol in fish are iono-osmoregulation and carbohydrate metabolism. Yet, our previous experiment showed that although injection of cortisol acetate significantly raised the levels of circulating cortisol in brook trout, plasma concentrations of glucose remained at control values, suggesting that cortisol alone could not have affected blood glucose levels in acid-stressed fish (Tam et al. 1987b). It is well known in mammals that hormones such as glucocorticoids, insulin, glucagon and the catecholamines are all directly involved in regulating energy metabolism. The aim of the present study is to test whether some of these hormones are also involved in energy metabolism in teleost fish during acid stress.

MATERIALS AND METHODS

The yearling brook trout used in this study were purchased from a commercial hatchery in January 1987. When the experiment was performed in June, the fish weighed 124±3(44) g. These were divided into 2 groups. One group was maintained as always in pH 7.83±0.01(16), while the pH of the other group was lowered to 4.50±0.05(8) with the addition of sulphuric acid. The fish were fed daily to near satiation with commercial trout food. The methods of fish maintenance, water dechlorination and acidification have been detailed in previous publications (Tam and Payson 1986; Tam et al. 1987b). The fish were either not treated, or injected i.p. with 0.1 mL 0.75% saline, insulin (bovine insulin; Sigma Chemical Co., St. Louis, Mo.; Insulin 1 = 0.5 i.u. per injection; Insulin 2 = 1.0 i.u. per injection) and metyrapone (3 mg per injection; Sigma Chemical Co.) while under tricaine methanesulphonate (Syndel Laboratories Ltd., Vancouver, B.C.) anaesthesia. Saline and insulin were injected daily on days 1, 3, 5 and 7, and metyrapone was injected twice (1000 and 1600 h) the day before acidification and once daily on days 1, 3, 5 and 7 of acid exposure. All fish were sacrificed on day 8. Plasma osmolarity was measured with an Osmette osmometer (Precision Systems; Framingham, Mass.). The methods for the determination of plasma glucose (Glucose Assay Kit; Fisher Scientific, Don Mills, Ont.), amino acids (colorimetry of the condensation products of the amino acids and 2,4,6-trinitrobenzene sulphonic acid), proteins (Folin phenol reagent), cortisol (radioimmunoassay) and liver glycogen concentrations (anthrone reagent) have all been described elsewhere (Tam et al. 1987a, b). Results are expressed as mean±SEM (number of observations). Significant differences between means were detected by one-way ANOVA and the Student-Newman-Keuls *a posteriori* test, and confidence limit was taken at the 5% level.

RESULTS AND DISCUSSION

As there was no significant difference between the results obtained from the untreated and saline injected fish, these results were pooled together as one control group. A considerable part of the stressful effect of the low environmental pH must have come from the significantly reduced plasma osmolarity (Fig. 1a). The major cause of the reduced osmolarity in brook trout has been shown to be sodium loss, which occurs instantly when the fish is introduced to acidic pH (Fryer et al. 1987). Cortisol secretion is usually stimulated under situations of stress. Firm evidence of increased cortisol secretion in acid-stress brook trout has been reported (Tam et al. 1987b). Interrenal cell hypertrophy and hyperplasia, and increased corticotrope activities begin soon after water acidification. Significant elevations in plasma cortisol concentrations have also been demonstrated during the first 4 d of acid exposure. After the initial stage, increased cortisol metabolic clearance rate probably occurs (Redding et al. 1984; Brown et al. 1986), and the elevated, but not significant increase in plasma cortisol levels recorded in the present study probably reflect the combined effects of increased secretory and clearance rates of the hormone (Fig. 1b). Plasma glucose levels of the untreated and saline injected fish maintained at pH 4.50 (control, Fig. 2a) are higher than, but not significantly different from those of the corresponding fish maintained at pH 7.83. This lack of significant difference contradicted our previous findings which showed that plasma glucose levels in brook trout increased significantly from days 4-8 to days 40-60 of exposure to pH 4.55 (Tam et al. 1987b). This discrepancy may be due to variations among groups of fish in their timing of response to stress and to the difference in experimental design, such as the use of tricaine and the very disruptive nature of the injection schedule in the present experiment. However, other indices of metabolism indicate that body reserves of the acid-stressed trout have been mobilized. Plasma amino acid levels have been significantly increased (Fig. 2b) and liver glycogen in acid-exposed fish has been significantly depleted (Fig. 2d). Metyrapone treatment lowers plasma amino acid concentrations in acid-stressed fish so that they are no longer significantly different from those of control fish kept at pH 7.83. Metyrapone is a proven agent that suppresses ACTH and stress-induced cortisol secretion (Leach and Taylor 1980). Thus, the stress-induced increase in cortisol secretion may have been one of the factors causing the plasma amino acid levels to increase. In this respect, brook trout differ from *Fundulus heteroclitus* in which cortisol has no influence on serum amino acid levels (Leach and Taylor 1980, 1982). Treatment with metyrapone at twice the dosage was also attempted and it appeared to be toxic, causing plasma cortisol levels to rise to 45 ng/mL and blood glucose concentrations to increase to 102 and 238 mg/dL respectively in neutral and acid-treated brook trout. Insulin treatment tends to lower blood glucose levels (Fig. 2a), and suppresses the stress-induced elevation of plasma amino acid concentrations. In either dosage used, plasma amino acid levels in the acid-treated fish are no longer significantly different from those of control fish maintained at pH 7.83 (Fig. 2b). Thus, besides cortisol, elevation of amino acid levels can also be affected by suppression of insulin secretion in acid-stressed brook trout. In fact, blockade of insulin secretion by corticosteroid has been observed in mammal (Barseghian and Levine 1980). None of the treatments appear to have altered the plasma protein concentrations (Fig. 2c). The exact cause of decrease in liver glycogen concentration in the insulin treated-pH 7.83 trout is uncertain (Fig. 2d). Food intake of these fish almost completely ceased during the treatment period and glycogen depletion could have been due to the lack of food intake.

Fig. 1. Plasma osmolarity (a) and cortisol concentration (b) in brook trout maintained at pH 7.83 (clear) and pH 4.50 (hatched). Control = untreated and saline injected. Insulin 1 and 2 = 0.5 i.u. and 1.0 i.u. insulin per injection. * = $p < 0.05$ compared with control group maintained at pH 7.83.

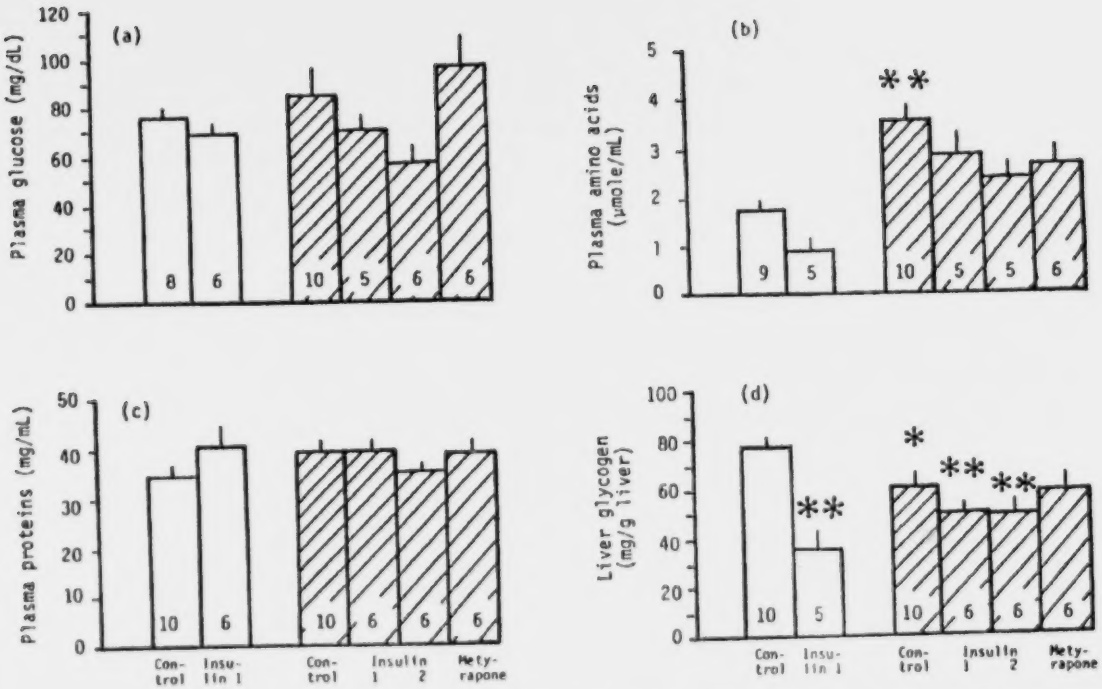

Fig. 2. Plasma levels of glucose (a), amino acids (b) and proteins (c), and liver glycogen concentrations (d) in brook trout maintained at pH 7.83 (clear) and pH 4.50 (hatched). Control = untreated and saline injected. Insulin 1 and 2 = 0.5 i.u. and 1.0 i.u. insulin per injection. * = $p < 0.05$ and ** = $p < 0.01$ compared with control group maintained at pH 7.83.

Other fish in the experiment fed normally. Although liver glycogen level is significantly lowered in the acid-stressed fish (pH 4.50 control, Fig. 2d), the stress-induced increase in cortisol secretion may not be responsible for this depletion. Both continuous stress and cortisol administration have been demonstrated to raise glycogen concentration in other teleost species (Leach and Taylor 1980, 1982). As reported in the literature (Ablett et al. 1981), insulin treatment does not have any effect on liver glycogen in the acid-stressed trout. The likely causes of liver glycogen depletion in acid-treated fish are therefore increased catecholamine and glucagon secretion. Enhanced secretion of catecholamines is a common response to stress (Mazeaud and Mazeaud 1981). Glucagon has been observed to stimulate glycogenolysis in eels (Chan and Woo 1978) and its secretion is enhanced by corticosteroid (Barseghian and Levine 1980). The results of this study raise the possibility that the extra energy needed to compensate for the adverse effects of acid stress may come from both the carbohydrate and protein reserves of the body and that the endocrine control of these metabolic changes is complex involving hormones such as cortisol, insulin, glucagon and the catecholamines.

ACKNOWLEDGEMENT

This research was supported by a Strategic Grant (G1569) awarded to W.H. Tam by the Natural Sciences and Engineering Research Council of Canada.

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UPTAKE AND DEPURATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY THE AMERICAN LOBSTER (*HOMARUS AMERICANUS*): RELATIONSHIP WITH TAINTING

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ABSTRACT

Lobsters (*Homarus americanus*) were exposed to a surface slick of a No. 2 fuel oil (single pulse) for 21 d and removed on days 3 and 4, 10 and 11, and 20 and 21. Hepatopancreas and muscle tissue were taken for PAH analysis and muscle tissue for organoleptic analysis which was carried out with triangle, paired-preference and odour tests. Concentrations of PAH in tissue and water were determined by HPLC with water sampling being carried out at 2, 4 and 8 h, and 1, 2, 3, 10 and 21 d.

There was no significant differences in taste between groups on days 3 and 4, or 20 and 21, but there was a significant difference in taste between groups on days 10 and 11 as well as a significant preference for the controls. At each of the three sampling times, the odour of the exposed samples differed significantly from controls.

Elevated levels of total PAH were found in the hepatopancreas of exposed lobsters on days 3 and 4, and 10 and 11. Levels returned to control values on days 20 and 21. Elevated levels of total PAH were found in the tail muscle on days 10 and 11 with levels approximating control values on days 3 and 4, and 20 and 21.

Observations support the hypothesis that tainting should drop in lobsters 1-2 wk after exposure to petroleum hydrocarbons.

RÉSUMÉ

Des homards (*Homarus americanus*) ont été exposés à une nappe de mazout numéro 2 (traitement en une seule fois) pendant 21 jours et des sujets ont été prélevés aux jours 3 et 4, 10 et 11, et 20 et 21. Du tissus hépatopancréatique et du tissu musculaire ont été prélevés pour le dosage des HAP et le tissu musculaire a été soumis aussi à un examen organoleptique constitué de tests en triangle, de préférence par paires et de tests pour l'odeur. Les HAP dans les tissus et l'eau ont été dosés par CLHP; les échantillons d'eau ont été prélevés à 2,4 et 8 h, ainsi qu'à 1, 2, 3, 10 et 21 jours.

Après les jours 3 et 4 ainsi que 20 et 21, il n'y avait pas différence significative de goût entre les groupes, mais il y avait une différance significative entre les groupes les jours 10 et 11, une préférence marquée étant affichée pour les témoins. Á chacune des trois périodes des prélèvement, l'odeur des échantillons exposés différait significativement de celle des témoins.

Des teneurs élevées de HAP totaux ont été trouvées dans l'hépatopancréas de homards exposés après 3 et 4 ainsi que 10 et 11 jours. Chez les sujets d'expérience prélevés à 20 et 21 jours, les concentrations prélevés à 20 et 21 jours, les concentrations avaient rejoint les valeurs observées chez les témoins. Des concentrations élevées de HAP totaux ont été trouvées dans le muscle de la queue aux jours 10 et 11, mais elles étaient approximativement égales aux valeurs trouvées chez les témoins aux jours 3 et 4 ainsi qu'aux jours 20 et 21.

Les observations confirment l'hypothèse à l'effet que la chair de homard devient moins altérée au goût au bout d'une semaine ou deux après exposition à des hydrocarbures.

UPTAKE AND DEPURATION OF TETRAETHYLLEAD BY RAINBOW TROUT

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ABSTRACT

Alkyllead compounds in the environment have been of much concern because of their high toxicity, bioaccumulation and wide-spread occurrence. In Canada, the major consumption of alkyllead compounds is tetraethyl-lead (Et4Pb) which is used as an antiknock additive in gasoline.

Exposure of rainbow trout (about 300 g) to 5 and 10 μg/L of Et4Pb (dissolved in methanol) caused high mortality to fish. Half of the fish (12) died within 15 d from loss of appetite, swimming disorientation, enlarged spleen and gall bladder and infection in the digestive tract. All control fish exposed to methanol survived. Analyses of Et4Pb in 11 tissues and organs of the dead fish revealed that brain contained the highest amount of Et4Pb (about 8 mg/Kg), followed by skin, intestine and visceral fat. A decrease in Et4Pb to 2.5 μg/L enabled the fish to survive. Uptake of Et4Pb from the water to the fish was very rapid and reached a steady state level after 7 d exposure. In this case, visceral fat of the fish contained the highest level of Et4Pb, followed by intestine and skin. The brain contained only 44 μg/Kg suggesting that the brain was the critical organ for Et4Pb toxicity.

Depuration of Et4Pb from exposed fish was initially very slow, followed by a faster decrease and eventually reaching residual levels. Hence concentrations of Et4Pb in fish from the environment would represent a balance between uptake and depuration of this compound in fish.

RÉSUMÉ

La présence dans le milieu de composés d'alkylplomb a suscité bien des inquiétudes parce que ce sont des composés fortement toxiques qui sont accumulés dans la pyramide alimentaire et dont la présence est générale. Au Canada, les composés alkylés de plomb sont principalement consommés sous forme de plomb tétraéthyle (Et4Pb), un produit anticognement ajouté à l'essence.

L'exposition de truites arc-en-ciel (environ 300 g) à des doses de 5 et 10 μg/L de Et4Pb (dissous dans le méthanol) a provoqué une forte mortalité chez ces poissons. La moitié des sujets (12) sont morts en présentant les symptomes suivants : perte d'appétit, désortientation dans la nage, rate et vésicule biliaire hypertrophiées et appareil digestif sous le coup d'une infection. Tous les témoins exposés au méthanol ont survécu. Les dosages d'Et4Pb pratiqués dans 11 tissus et organes des poissons qui sont morts ont montré que l'Et4Pb s'est logé en plus grande quantité dans le cerveau (environ 8 mg/kg), ensuite dans la peau, l'intestin et le tissus adipeux des viscères. Une diminution de la concentration jusqu'à 2.5 μg/L a permis au poisson de survivre. L'absorption d'Et4Pb par les poissons s'est faite très rapidement et a atteint un plateau après 7 jours d'exposition. Dans ce cas, le tissu adipeux des viscères contenait la plus forte teneur en Et4Pb, et il était suivi par l'intestin et la peau. Le cerveau ne contenait plus que 44 μg/kg, ce qui semble indiquer que le cerveau constituait l'organe critique pour ce qui est de la toxicité de l'Et4Pb.

La dépuration chez les poissons exposés a d'abord été très lente pour s'accroître et porter les concentrations au niveau résiduaire. Ainsi, la concentration d'Et4Pb chez les poissons à l'état sauvage correspondrait à un équlibre entre l'assimilation et la dépuration chez les poissons.

DEPRESSED MERCURY LEVELS IN BIOTA FROM ACID STRESSED LAKES NEAR SUDBURY, ONTARIO

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ABSTRACT

Some recent studies have suggested that elevated Hg levels in fish are a result of lake acidification. The purpose of this study was to determine if negative relationships between lake pH and fish Hg content, which have been reported for several areas, are consistent over a wide range of lake conditions. Samples of crayfish, fish, mink and otter from different sites in Ontario, Canada, were analyzed for total Hg content. Concentrations of Hg were lowest in specimens from the most acid-stressed watershed near Sudbury. Crayfish Hg levels displayed a positive correlation with lake pH. Crayfish and perch Hg levels increased in a linear fashion with distance away from Sudbury. We suggest that selenium, and possibly other heavy metals, emitted from the smelters are inhibiting Hg methylation in lakes near Sudbury. The data support the premise that pH is not the sole factor affecting Hg uptake by freshwater biota.

RÉSUMÉ

Certaines études récentes ont indiqué que la concentration élevée en Hg chez le poisson résulte de l'acidification des lacs. Cette étude a pour but de déterminer si les rapports inverses entre le pH de l'eau des lacs et la concentration en Hg dans le tissu des poissons, rapportés dans plusieurs secteurs, se vérifient à l'intérieur d'une vaste plage de conditions des lacs. Des écrevisses, poissons, visons et loutres ont été capturés dans différentes régions de l'Ontario au Canada et la teneur en Hg total de leurs tissus a été mesurée. Les concentrations les plus faibles en Hg ont été trouvées dans les sujets capturés à l'intérieur du bassin versant le plus stressé par les pH acides, près de Sudbury. La concentration en Hg des écrevisses était en corrélation positive avec le pH du lac. Celle-ci et la concentration chez la perche augmentaient linéairement avec l'éloignement de Sudbury. Nous avançons l'hypothèse à l'effet que le sélénium et peut-être d'autres métaux lourds dégagés par les hauts-fourneaux inhibent la méthylation du Hg dans les lacs à proximité de Sudbury. Les résultats confirment l'hypothèse selon laquelle le pH ne constitue pas le seul facteur qui agit sur l'absorption du Hg dans les biotes d'eau douce.

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WORKSHOP PROCEEDINGS

The Proceedings of the Annual Aquatic Toxicity Workshops have been published as a series of technical reports listed below. Copies of recent Proceedings are available from the Continuity Chairman, Aquatic Toxicity Workshop, Bayfield Institute, Canada Centre for Inland Waters, Burlington, Ontario, Canada, L7R 4A6. Copies of most Proceedings are available for a charge from Micromedia Limited, 165 Hotel de Ville, Place du Portage, Hull, Quebec, J8X 3X2, (819) 770-9928. Their catalog numbers (MLCN) are listed.

Proceedings of the Thirteenth Annual Aquatic Toxicity Workshop: November 12-14, 1986, Moncton, New Brunswick. Edited by J.S.S. Lakshminarayana. Can. Tech. Rep. Fish. Aquat. Sci. 1575: 178 p. (MLCN:88-01709)

Proceedings of the Twelfth Annual Aquatic Toxicity Workshop: November 5-8, 1985, Thunder Bay, Ontario. Edited by G.W. Ozburn. Can. Tech. Rep. Fish. Aquat. Sci. 1462: 229 p. (MLCN: 86-5828).

Proceedings of the Eleventh Annual Aquatic Toxicity Workshop: November 13-15, 1984, Vancouver, British Columbia. Edited by G.H. Geen and K.L. Woodward. Can. Tech. Rep. Fish. Aquat. Sci. 1480: 330 p. (MLCN: 87-1493).

Proceedings of the Tenth Annual Aquatic Toxicity Workshop: November 7-10, 1983, Halifax, Nova Scotia. Edited by P.G. Wells and R.F. Addison. Can. Tech. Rep. Fish. Aquat. Sci. 1368: 475 p. (MLCN: 86-1103).

Proceedings of the Ninth Annual Aquatic Toxicity Workshop: November 1-5, 1982, Edmonton, Alberta. Edited by W.C. Mckay. Can. Tech. Rep. Fish. Aquat. Sci. 1163: 243 p. (MLCN: 84-3262).

Proceedings of the Eighth Annual Aquatic Toxicity Workshop: November 2-4, 1981, Guelph, Ontario. Edited by N.K. Kaushik and K.R. Solomon. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 255 p. (MLCN: 83-2515).

Proceedings of the Seventh Annual Aquatic Toxicity Workshop: November 5-7, 1980, Montreal, Quebec. Edited by N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert, and M. Speyer. Can. Tech. Rep. Fish. Aquat. Sci. 990: 519 p. (MLCN: 82-0070).

Proceedings of the Sixth Annual Aquatic Toxicity Workshop: November 6 & 7, 1979, Winnipeg, Manitoba. Edited by J.F. Klaverkamp, S.L. Leonhard, and K.E. Marshall. Can. Tech. Rep. Fish. Aquat. Sci. 975: 291 p. (MLCN: 81-1492).

Proceedings of the Fifth Annual Aquatic Toxicity Workshop: November 7-9, 1978, Hamilton, Ontario. Edited by P.T.S. Wong, P.V. Hodson, A.J. Niimi, V. Cairns, and U. Borgmann. Fish. Mar. Ser. Tech. Rep. 862: 342 p. (MLCN: 80: 4061).

Proceedings of the Fourth Annual Aquatic Toxicity Workshop, November 8-10, 1977, Bayshore Inn, Vancouver, B.C. Edited by J.C. Davis, G.L. Greer, and I.K. Birtwell. Fish. Mar. Ser. Tech. Rep. 818: 211 p. (MLCN: 80: 4022).

Proceedings of the Third Annual Aquatic Toxicity Workshop, November 2-3, Halifax, Nova Scotia. Edited by W.R. Parker, E. Pessah, P.G. Wells, and G.F. Westlake. Environ. Prot. Ser. Tech. Rep. EPS-5-AR-77-1.

Proceedings of the Second Annual Aquatic Toxicity Workshop, November 4-5, 1975. Rexdale, Ontario. Edited by G.R. Craig. Ontario Ministry of the Environment.

Compendium of Aquatic Toxicity Studies in Canada. 1974. Unpublished Report, Freshwater Institute, Winnipeg, Manitoba.